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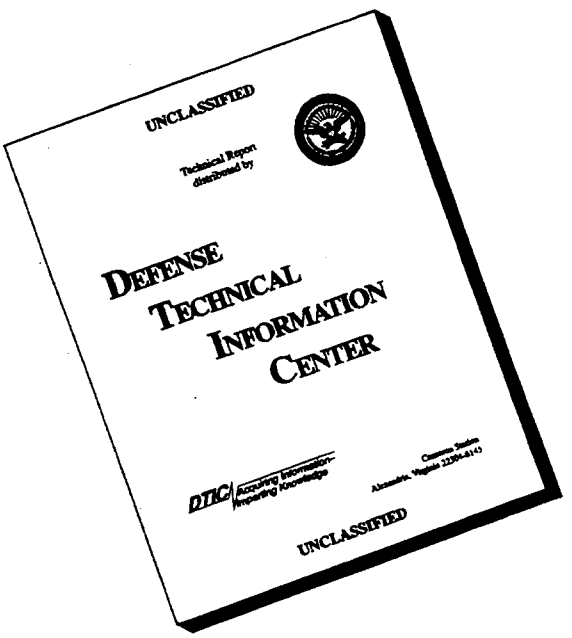

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13. ABSTRACT *(Maximum 200*

A prototype instrument and associated sterile disposable tube sets for the deglycerolization of frozen red blood cells (RBC) were built and tested. The instrument is capable of prediluting and washing up to two units of blood per tube set. Our results show that when the performance of our machine is compared to centrifugation we obtain equivalent glycerol removal. Further intracellular Na^+ and K^+ of the washed RBCs is acceptable. Our RBC recovery is low (about 70% versus about 85% using centrifugation) and the supernatant haemoglobin concentration of the washed blood high (200 mg dL^{-1} versus about 100 mg dL^{-1} using centrifugation).

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Franco Cattivo 3/10/96

PI - Signature Date

Contents

Annual Report April 1996

Feasibility Assessment of a Structurally Closable, Automatable Technique for Deglycerolization of Frozen RBC (Human) prepared by Ranil Wickramasinghe, Ph.D.

1.0 Introduction

Frozen red blood cells have a shelf life of at least 10 years. This makes them particularly suitable in times of war since they can be strategically deployed in appropriately located depots. During the freezing process glycerol is added as a cryoprotective agent to avoid cell damage [1]. After thawing and before transfusion the concentration of glycerol has to be reduced to biocompatible levels (from 1.57 M to less than 0.1 M).

Freezing and thawing red blood cells leads to haemolysis of the older more fragile cells [2]. This in turn leads to an increase in the supernatant haemoglobin concentration which before transfusion must be reduced to less than 200 mg dL^{-1}.

Finally the washed blood has to be concentrated. After thawing the glycerol concentration in the red blood cell concentrate is reduced by adding solutions of decreasing osmolalities (predilution) until physiological saline can be used for washing without damaging the cells [1]. After predilution the haematocrit of the blood is usually in the range 10-20%. The washed unit has to be concentrated to a haematocrit of over 40%.

At present the glycerol and supernatant haemoglobin concentrations are reduced to biocompatible levels and the blood is concentrated by a combination of manual and semi-automatic operations [1]. Cell washing is accomplished with a batch centrifugation process using a Haemonetics Cell Washing System 115 (Haemonetics, Braintree, MA). The process takes about 45 minutes and requires 1.5 L of saline.

Though red blood cells washed with the Haemonetics system meet the required criteria for transfusion there are a number of disadvantages with the procedure. Due to the presence of a rotating seal, the system is not closed to the atmosphere and is thus open to contamination. Consequently the post-thaw shelf life of the blood is only 24 hours. In times of war, this leads to many logistical problems as well as wasted units. The system is large (9.1 ft^3), heavy (154 lbs), floor mounted and labour intensive requiring trained operators. These requirements are particularly onerous on the military.

In phase I of the current SBIR program we showed the feasibility of replacing centrifugation with membrane filtration to achieve a structurally closed system capable of concentrating and washing the thawed blood. The process was optimized by minimizing both the processing time and diluent volume required. We showed that an optimum haematocrit exists which minimizes the time taken to wash a unit of blood. Further we showed that an optimized hollow fibre module requires fibres of around 200 μm ID. Our phase I results were published in Journal of Membrane Science 110 (1996) 169-180. A copy of the paper is included in Appendix I.

During the first year of the current contract we have built an instrument capable of washing frozen red blood cells. The instrument was designed based on the data obtained in phase I. (In phase I a Trio instrument was used to wash frozen red blood cells. The Trio is described in the phase I annual report). Here we report on the prototype instrument designed and built during the first year of the current contract. Software and tube set development are also described. Experimental results obtained to date are presented. Our progress is compared to that originally proposed in the phase II proposal.

All references cited in this report are given in Appendix 2. Information for the prototype instrument design and manufacture was provided by McClellan Inc. Their original report is included as Appendix 3. Ann Harris, our software consultant submitted a report on her activities throughout the year. It is included as Appendix 4. Jon Alt's report on the design and manufacture of the sterile tube set is included as

Appendix 5. In writing this report I have drawn from all of these sources. The report and Appendices 1 and 2 are consistent. Appendices 3-5 are included for reference if the reader is interested in more details of various aspects of the development of the prototype instrument, software and sterile tube set.

2.0 Prototype Instrument

During the first three months of the contract, (15 Mar to 15 Jun 1995) schedules were developed covering all of the design and related technical activities for the first contract year. A preliminary design specification for the system was also developed. All documentation available for the Trio was handed over to McClellan Inc. It was decided that the prototype instrument would make use of as many of the Trio components as possible. The purpose of the prototype instrument would be to complete both the predilution and washing of frozen red blood cells in an automated fashion as described in the Phase II proposal. The data obtained from testing the prototype instrument would be used to design the pre-production unit we plan to build in the second year of the contract.

During the second quarter of the contract process changes resulted in many modifications to both the CAD design layouts and to the system specification. The design and build of two foamcor product mock-ups greatly facilitated the timely resolution to numerous problems in the tube set design.

It soon became evident that further optimization of the washing process using the Trio was of little value. The tube set being developed for the prototype machine could not be simulated using the Trio tube set. Therefore, the delivery date of the prototype was advanced significantly. The final product specification was agreed to by the team on 12 July 1995. The delivery date for the prototype instrument was advanced by almost seven weeks. During the assembly and debugging of the prototype problems were identified and quickly corrected. The Trio power supply proved inadequate and was replaced.

To date the following items have been identified as important issues that need to be addressed in the pre-production unit:

- product size and ship board requirements;
- integrated shaker table design;
- source compact peristaltic pumps;
- refine instrument and tube set interface;
- define simple operator control panel.

A preliminary plan for the design and build of the pre-production prototype system has been prepared. Foamcor mock-ups of the new design will be fabricated for evaluation and the operator interface studied. The plan requires starting the preliminary selection of design components and visiting a ship by mid April 1996. The prototype will then be built by early November, 1996 and be ready for performance testing by the end of December 1996.

2.1 Prototype Instrument Software

The prototype instrument software was based on Hemasure's Trio instrument clarification software. The clarification process was modified to perform the entire blood wash process: predilution of the thawed blood, loading of the prediluted blood into the processing bag, washing and pumping the washed blood into the collection bag. The prototype instrument includes a simplified version of the Trio operator interface and diagnostic functions.

In order to have the blood wash software as complete as possible when the prototype was ready for testing, process development was performed using the Trio instrument. All blood wash process steps were successfully simulated and step parameters (flow rates, volumes, etc.) tentatively selected. This testing took place throughout the summer of 1995.

During September and October 1995, the blood wash program developed on the Trio instrument was adapted for the new prototype instrument built by McClellan Inc. The instrument and program were debugged at McClellan's manufacturing facility before shipment to the Naval Blood Research Laboratory (Boston, MA) in November.

At this time the prototype is being used to wash actual units of blood in order to verify the process. Due to the need to easily modify the process at this stage of testing, parameter editing and instrument diagnostic functions have been retained from the TRIO software. When this testing phase is complete, the process will be reevaluated and modified for optimization of blood quality, recovery rate, and processing time.

2.2 Disposable Set

2.21 Module development

Hollow fibres from AKZO (Wuppertal, Germany), Mitsubishi Rayon America Inc. (New York, NY) Microgon (Laguna Hills, CA) as well as our own were evaluated (see second quarterly report). It was decided to use Hemasure fibres as we could control the manufacturing process and thus optimize the fibres for glycerol removal from frozen red blood cells. Specifically we were interested in minimizing the recirculation rate, maximizing the permeate flux and minimizing haemoglobin rejection. Polyethersulphone hollow fibres with an outside diameter of 450 μm and an inside diameter of about 250 μm were selected. The fibres were coated hydroxypropyl cellulose in order to increase their hydrophillicity.

2.22 Encapsulation of Fibres

In accordance with the results of phase I, 2,000 fibres were potted in housings which provided an active fibre length of 8.0 cm. This gave a membrane surface area of 1260 cm^2. The modules were encapsulated using a polyurethane for medical applications provided by CasChem (Bayonne, NJ). A number of experiments were conducted to obtain optimum conditions for encapsulation of the modules.

After encapsulation, the hollow fibre modules required further processing. The ends of the encapsulated fibres were inspected to ensure the fibres were not damaged. The end caps were then welded and the complete module again integrity tested to ensure there were no broken fibres.

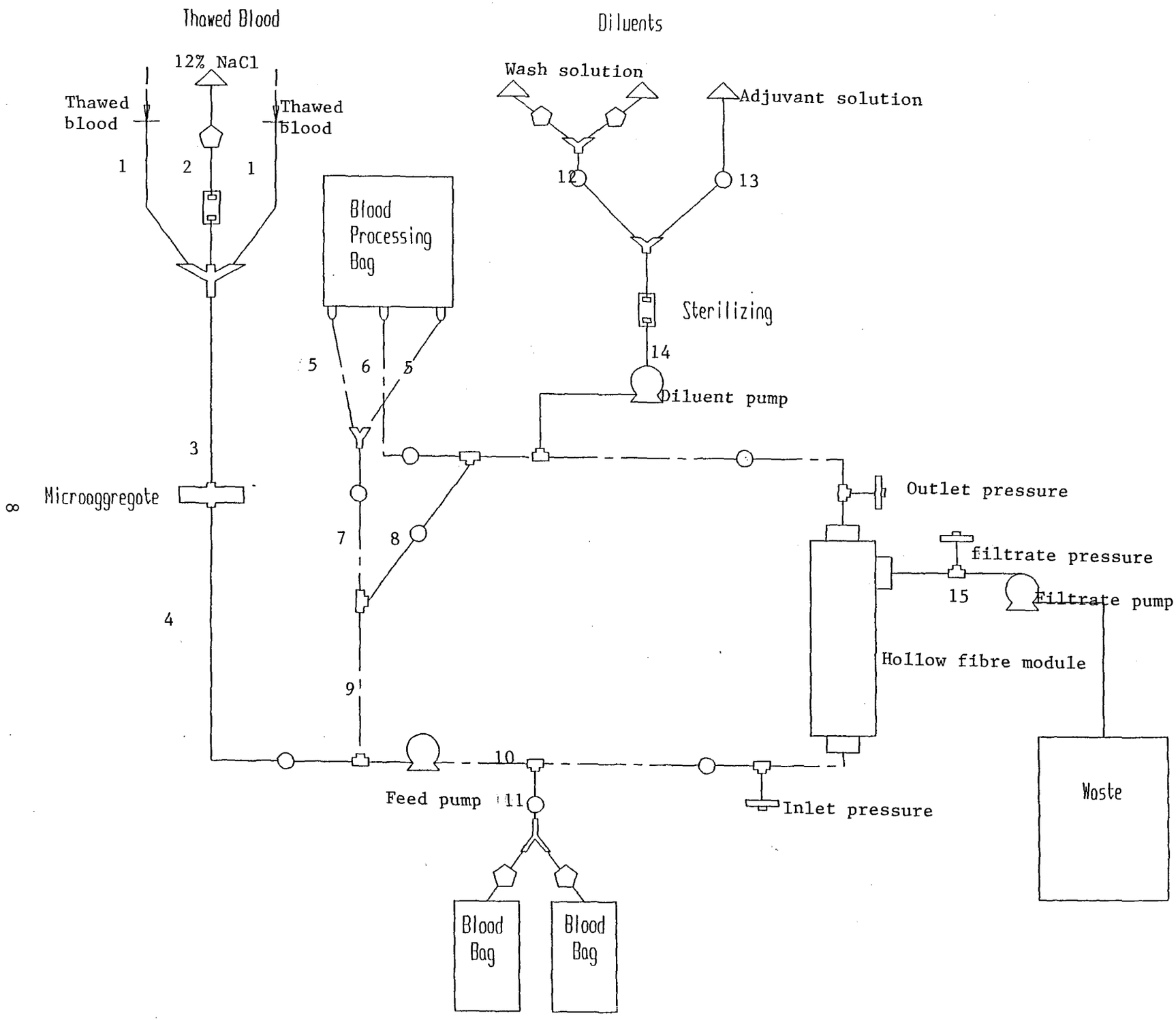
2.23 Tube set

To ensure the tube set would be compatible with the prototype instrument the foamcor model was used as a mock up instrument. All components for the disposable tube set were selected so that they would be stable under gamma irradiation. Sterilizing grade and microaggregate filters were chosen appropriate for the flow rates to be used.

The processing bag made of PVC contained two baffles. The bag had three ports: two outlet ports on either side of the baffle and an inlet port in the centre of the bag. A 5 L waste bag was necessary to hold the waste produced from washing two units of frozen blood.

3.0 Experimental Results

Figure 1 is a schematic drawing of the tube set. A unit of thawed blood was attached by sterile docking to either of the lines labelled 1. The blood was then placed on a shaker and while the shaker was on 50 mL of 12% NaCl solution was added. The addition of this solution was by gravity flow and was the only manual step in the entire process. In accordance with the protocol developed at the Naval Blood Research


Thawed Blood
Diluents
12% NaCl
Thawed blood
Thawed blood
Wash solution
Adjuvant solution
1
2
1
12
13
Blood Processing Bag
Sterilizing
14
5
6
5
Diluent pump
3
Microaggregate
Outlet pressure
7
8
filtrate pressure
15
Filtrate pump
4
Hollow fibre module
9
10
Feed pump
11
Inlet pressure
Waste
Blood Bag
Blood Bag

Laboratory (Boston, MA), the shaker was switched off for two minutes after addition of the 12% NaCl solution. During this time, wash solution was pumped from line 12 into the processing bag. Operation of the shaker was regulated by the controller.

Next 100 mL of wash solution was pumped into the thawed blood bag. Again the shaker was turned off for two minutes after addition of the wash solution. During this time the processing loop (lines 5,7,9,10, hollow fibre module and line 6) were primed by pumping the wash solution in the processing bag around the loop using the feed pump. After two minutes, a further 110 mL of wash solution was added to the thawed blood. Again for two minutes the shaker was turned off and the feed loop primed.

Eventhough we coated the surface of our hollow fibres to increase their hydrophillicity, it was felt that excessive haemolysis could occur if red blood cells were to contact air in the pores of the membrane. Further air trapped in the membrane pores could lead to a significant decrease in permeate flux. Thus it was decided to prime the loop and module during predilution of the blood.

After completion of the predilution steps, the thawed blood was pumped into the processing bag via lines 3,4,10, hollow fibre module, and line 6 (load step). The blood was then concentrated, washed and further concentrated to a haematocrit of around 40%. The processed blood was then pumped via lines 5,7,9,10 and 11 into a blood bag. The final step consisted of a gravity drain step to recover blood trapped in lines 6 and the hollow fibre module. If a second unit was to be processed it was then attached to the unused line segment labelled 1 and the above process repeated.

A number of units of frozen blood were used to debug the process. Once the system was operating properly, a testing plan was devised in conjunction with Dr. Valeri of the Naval blood Research Laboratory. Eight units of blood were washed using eight tube sets and a further four units using two tube sets. The purpose of the testing was to determine if we could obtain washed blood of equal quality to that obtained from the Haemonetics Cell Washer 115. The results are given in Table 1.

In Table 1 the date of washing (test date) and blood unit number are given in the first two rows. Next pre-wash blood weight, haematocrit, intracellular Na^+ and K^+, supernatant haemoglobin and osmolality are shown. The values of these parameters after washing are given in the next 5 rows. The last four rows of the table contain the wash time (predilution and washing), waste volume and RBC recovery. RBC recovery is determined using the waste and pre-post methods (Valeri, 1996)

Comparing the pre-post data we see that for all units the final glycerol concentration is less than 350 mOsm/kg similar to the results obtained from the Haemonetics Cell Washer 115. Further post-wash intracellular Na^+ and K^+ are within the expected range (Valeri, 1996). However after washing the supernatant haemoglobin is always in excess of 200 mg dL^{-1} where as with the Haemonetics Cell Washer it is around 100 mg dL^{-1}. Further our RBC recoveries are generally between 60 and 70% as opposed to about 85% obtained from the latter machine.

The duration of the washing process is about 105 minutes, significantly longer than 45 minutes taken by the Haemonetics machine. Waste volumes are about 1800 mL. We used about 2.2 L of wash solution as opposed to 1.5 L by the Haemonetics machine. The waste volume is always a little less than the total volume of diluent added since the haematocrit of washed blood is always a little lower than that of the thawed blood.

Since the purpose of the first series of experiments was to obtain performance similar to the Haemonetics machine it was evident that modifications to the washing process were needed. Upon review of the experimental data with Dr., Valeri it was decided to focus on the low RBC recovery. The close agreement between the two methods of determining recovery means few red blood cells are retained by the tube set. Thus low recovery must be due to high haemolysis. If the level of haemolysis could be reduced supernatant haemoglonbin will also be reduced. It was felt that reducing the wash time and wash solution volume used could be addressed once RBC recovery was improved.

We believed our low blood recoveries were due to excessive haemolysis during predilution. As stated, the module was primed in order to protect against possible haemolysis due to blood contacting air in the membrane pores. However this results in a small volume of high osmolality blood contacting a large

SBIR Blood Cell Wash Test Data Summary I

Identification:												
Test Date	1/29/96	1/29/96	2/6/96	2/6/96	2/13/96	2/13/96	2/20/96	2/20/96	2/27/96	2/27/96	2/28/96	2/28/96
Blood Unit ID #	9401076	9401419	9502414	9502416	9502386	9502384	9401006	9400971	9401357*	9401369*	9401012**	9401026**
Pre Wash Data:												
Blood Weight (g)	275	407	289	413	391	458	407	332	413	358	268	404
Hematocrit (%)	63	58	62	55	57	47	56	57	54	56	70	59
Intracellular NA+ (mEq/10E+12 RBC)	n/a	n/a	3.31	3.28	4.09	3.35	2.22	3.17	2.53	3.22	2.62	2.67
Intracellular K+ (mEq/10E+12 RBC)	5.20	3.76	6.50	5.82	6.75	5.31	7.21	5.16	6.51	6.52	7.13	6.16
Supernatant Hb (mg/dL)	875	1000	918	828	413	346	505	935	755	734	1187	1022
Osmolality (mOsm/kg H20)	5604	5166	5586	5196	4660	4660	4860	4900	4960	4760	5300	4740
Post Wash Data:												
Hematocrit (%)	41.0	48.5	41.0	36.0	42.0	33.5	29.5	38.0	39.0	51.0	37.8	46.0
Intracellular NA+ (mEq/10E+12 RBC)	n/a	n/a	2.43	2.27	2.45	2.42	1.34	2.45	1.52	2.42	1.43	1.63
Intracellular K+ (mEq/10E+12 RBC)	5.00	4.97	5.49	4.75	5.17	3.99	4.57	4.00	4.70	5.42	5.35	4.17
Supernatant Hb (mg/dL)	253	440	223	144	252	252	261	335	458	267	235	568
Osmolality (mOsm/kg H20)	307	322	329	317	320	320	318	322	343	346	309	324
Wash Data:												
Duration (minutes)	n/a	n/a	83	143	88	89	119	82	119	102	89	113
Waste Vol (mL)	1400	1500	1400	1400	2300	2200	2000	1400	1400	1300	1400	1400
Recovery Waste (%)	73	74	78	74	77	68.8	63.59	72.15	71.3	78.5	50	79.2
Recovery Pre Post (%)	65	68.8	71	71	70	63.5	60.63	65.49	64.4	77.8	48	74.3

* Both units washed with one tubeset.
** Both units washed with one tubeset.
n/a Not Available.

volume of wash solution in the processing bag during the commencement of the load step. This could cause haemolysis. Thus it was decided not to prime the module during predilution. Some haemolysis was expected when the blood contacted air in the membrane pores. However if the haematocrit of the blood is low enough this could be minimized. Further if the haematocrit of the blood was sufficiently low, the supernatant solution should be able to adequately wet the membrane and displace trapped air, thus ensuring no reduction in permeate flux due to improper wetting of the fibres.

The protocol for washing was modified to delete all priming steps. As before a unit of thawed blood was sterile docked to the tube set. The blood was then placed on a shaker and while the shaker was on 50 mL of 12% NaCl solution was added. In accordance with the protocol developed at the Naval Blood Research Laboratory (Boston, MA), the shaker was switched off for two minutes after addition of the 12% NaCl solution. Next 100 mL of wash solution was pumped into the thawed blood bag. Again the shaker was turned off for two minutes after addition of the wash solution. After two minutes, a further 150 mL of wash solution was added to the thawed blood. Again for two minutes the shaker was turned off. Finally another 100 mL was added to the thawed blood while the shaker was running. Then the shaker was switched off for two minutes. After completion of these predilution steps, the thawed blood was pumped into the processing bag and the process continued as before.

It was decided to wash four units of blood with four tube sets using the modified protocol. At the end of March two of these units had been washed. The results are given in Table 2. The Table format is the same as for Table 1. For the unit 3401347, the RBC recovery was 80%. However the supernatant haemoglobin concentration was still high, before washing 819 mg dL^{-1}, after washing 393 mg dL^{-1}. For unit 9501753 the recovery was only 60%. However the amount of supernatant haemoglobin removed was much greater than for the first unit (initial supernatant haemoglobin 1544 mg dL^{-1} final supernatant haemoglobin 460 mg dL^{-1}). The results are encouraging but inconclusive. The remaining two units should be washed and the data base increased.

Our testing to date shows that glycerol removal and intracellular Na^+ and K^+ are similar to the results obtained by the Haemonetics Cell Washer 115. However our blood recoveries are much lower and the supernatant haemoglobin concentration is much higher. We believe the two are linked. When we remove all the module priming steps from the process we appear to obtain better RBC recoveries.

4.0 Program Schedule

Exhibits, E1-1, E2-1 and E3-1 were prepared for the phase ii proposal. Exhibit E1-1 shows that at this time we have completed all proposed tasks relating to red cell washing and have begun obtaining test results using the prototype instrument. This latter task was to have commenced in the fifth quarter of the contract. The table also shows that process development for platelet washing was to have been completed. At this time 5 units of frozen platelets have been washed. The platelets are washed in a pH 5.0 solution. Platelet recoveries were around 80%. However as platelets are deactivated at this pH we are unable to determine their viability. We hope to meet Dr. Valeri and determine a possible alternative test for platelet viability and then wash two more units of frozen platelets. The complete results for platelet washing will then be presented in a subsequent report.

The prototype instrument was delivered ahead of schedule (see Exhibit E2-1). At the end of March about 30 of the sterile, disposable tube sets had been fabricated. However completion of the remaining 20 disposable sets over the next month will not affect overall progress. As already stated testing of the prototype instrument with frozen red blood cells has already commenced. All quarterly reports were submitted on time. Upon discussions with the military, review meetings (see Exhibit E3-1) were not considered necessary.

SBIR Blood Cell Wash Test Data Summary II

Identification:		
Test Date	3/18/96	3/21/96
Blood Unit ID #	9401347	9501753
Pre Wash Data:		
Blood Weight (g)	438	456
Hematocrit (%)	51	51
Intracellular NA+ (mEq/10E+12 RBC)	2.49	2.43
Intracellular K+ (mEq/10E+12 RBC)	6.08	3.68
Supernatant Hb (mg/dL)	819	1544
Osmolality (mOsm/kg H20)	5120	4700
Post Wash Data:		
Hematocrit (%)	40.5	31.0
Intracellular NA+ (mEq/10E+12 RBC)	1.83	2.25
Intracellular K+ (mEq/10E+12 RBC)	4.84	4.00
Supernatant Hb (mg/dL)	314	460
Osmolality (mOsm/kg H20)	393	367
Waste Data:		
Duration (minutes)	90	99
Wash Vol (mL)	1600	1700
Recovery Waste (%)	82.64	70
Recovery Pre Post (%)	80.54	68

EXHIBIT E1-1
PROGRAM SCHEDULE, DEVELOPMENT

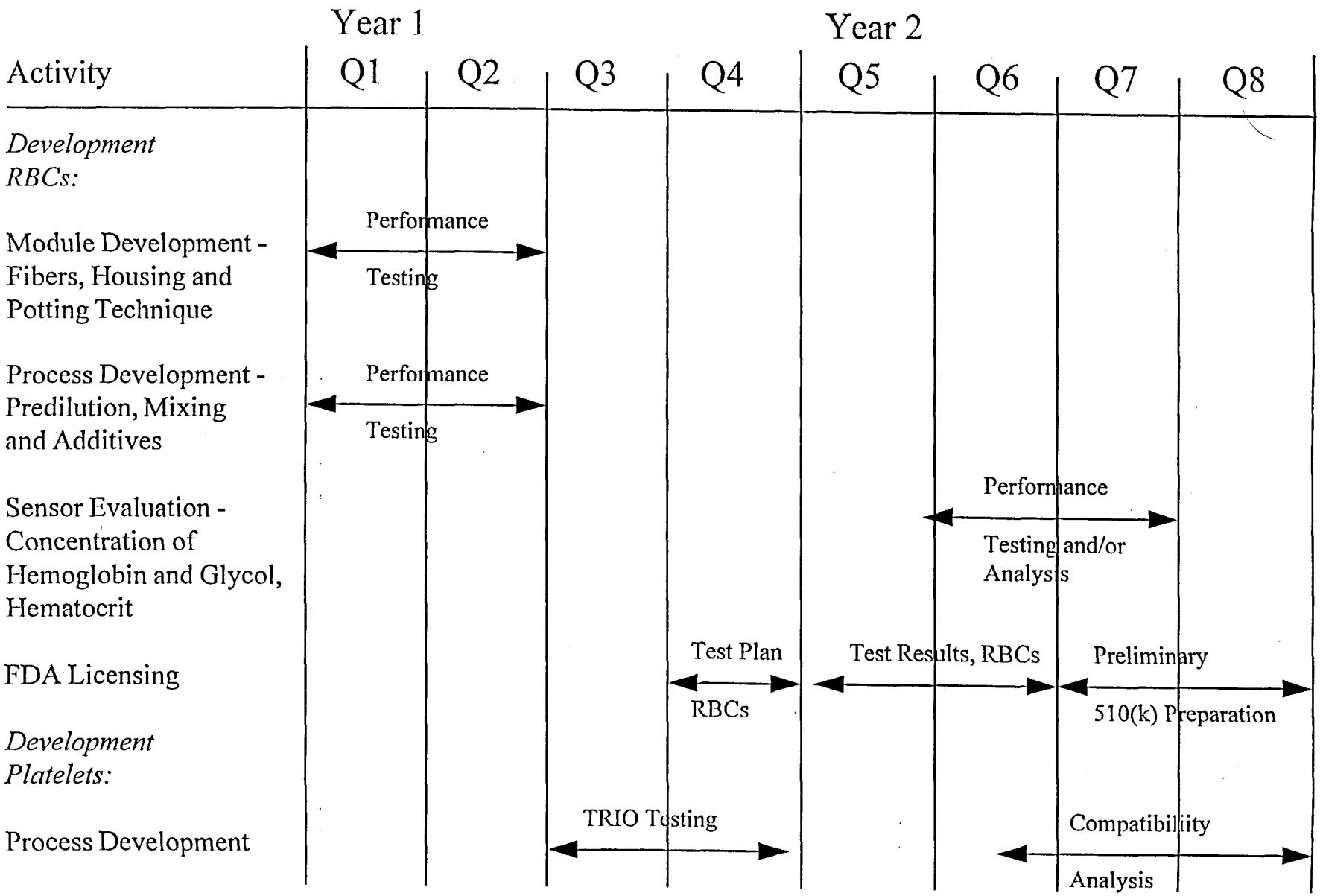


EXHIBIT E2-1
PROGRAM SCHEDULE, DELIVERABLES

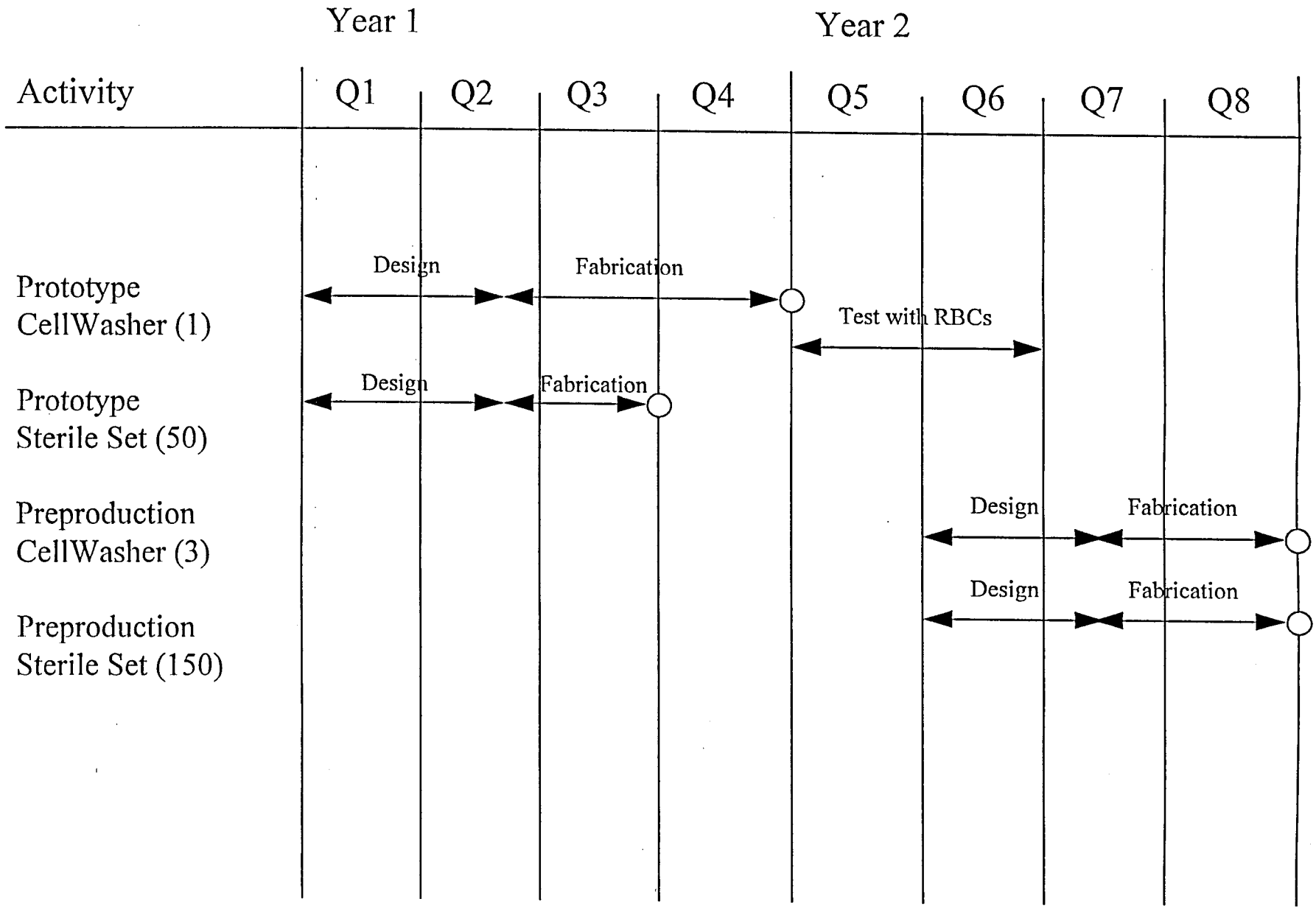
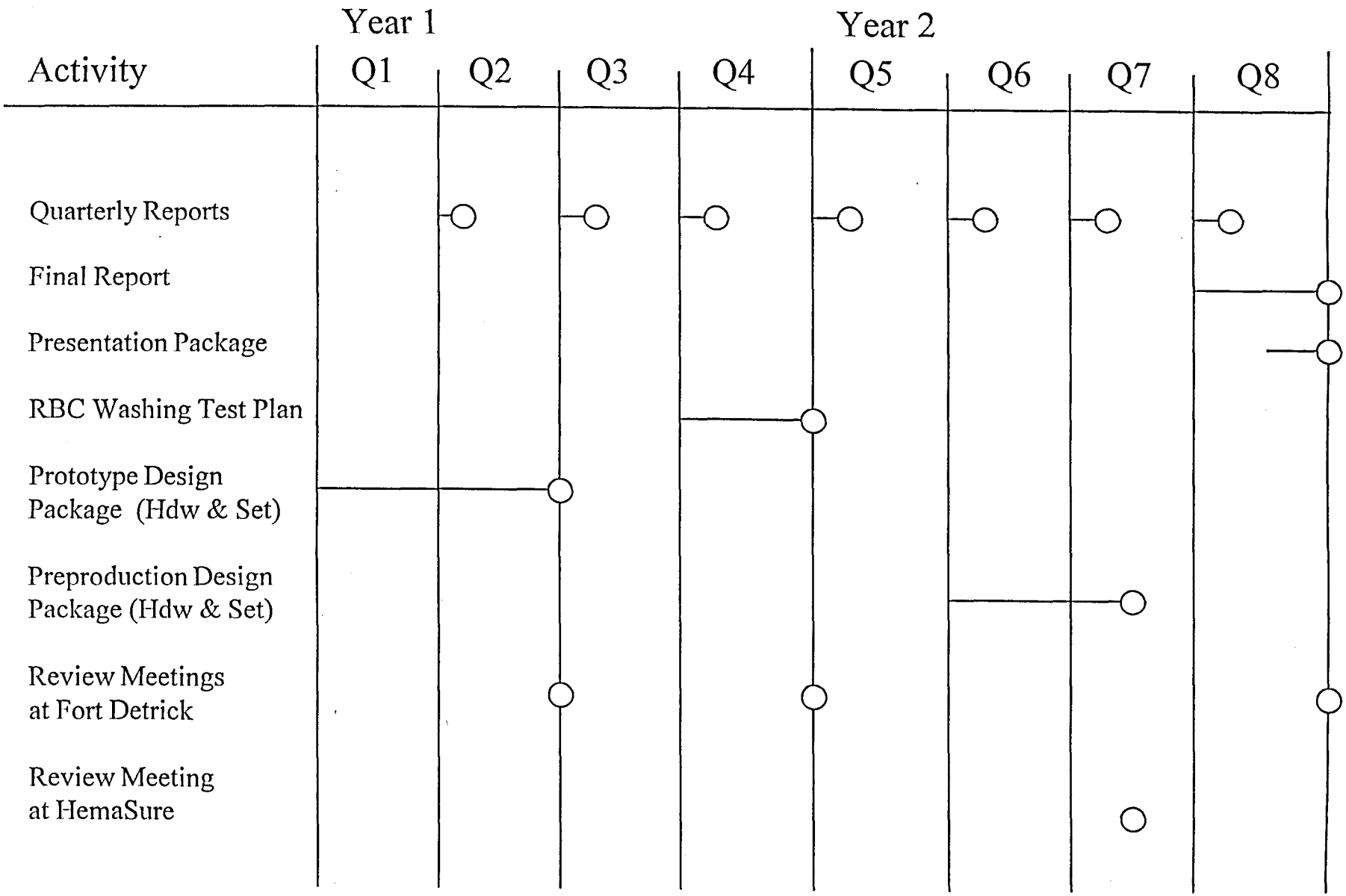


EXHIBIT E3-1
PROGRAM SCHEDULE, PROGRAM MANAGEMENT



5.0 Conclusions

Our work in year one of the contract can be summarized as follows:

* Development and manufacture of an automated prototype instrument for washing thawed RBCs was completed ahead of schedule.

* Washing 12 units of blood with the prototype instrument resulted in lower RBC recoveries and higher supernatant haemoglobin concentration than from the Haemonetics Cell Washer 115.

* The washing protocol was modified in order to improve RBC recovery. Testing of the modified protocol is not yet complete.

* Platelets have been washed using the Trio instrument. The results will be presented in a subsequent report.

Appendix 1 Washing frozen red blood cell concentrates using hollow fibres

ELSEVIER

Journal of Membrane Science 110 (1996) 169–180

journal of MEMBRANE SCIENCE

Washing frozen red blood cell concentrates using hollow fibres [1]

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Received 21 March 1995; revised 10 August 1995; accepted 31 August 1995

Abstract

Today the cryopreservation of human blood products is routine. However, before reinfusion the cryoprotectant, often glycerol, has to be removed. We have designed a combined microfiltration diafiltration process using microporous hollow fibres for removing glycerol from frozen red blood cell concentrates. As the system can be closed to the atmosphere there is no possibility of infection of the "washed" blood. Thus the post-thaw shelf life of the blood may be greatly increased. The process has been optimized by minimizing both the processing time and diluent volume required. Finally a hollow fibre module capable of completing the entire washing process in 30 min has been developed. We show that such a module requires hollow fibres with an inside diameter of 200 μm. The design equations we present are generally applicable to the design of hollow fibre microfiltration systems.

Keywords: Diafiltration; Deglycerolization; Microfiltration; Microporous and porous membranes; Modules

1. Introduction

Blood products are essential for the resuscitation of causalities. Liquid preserved whole blood and red blood cell concentrates have a very short shelf life. On the other hand frozen red blood cells have a shelf life of at least 10 years. This makes them particularly suitable in times of war since frozen red blood cells can be strategically deployed in appropriately located depots. Unlike liquid preserved blood from the mainland United States no complicated logistic manoeuvres associated with collection, testing, inventory and transshipment are required. As the units are all type O or universal blood, no cross-matching is necessary.

Before freezing the red blood cells, glycerol is added as a cryoprotectant to avoid cell damage [1,2]. However before transfusion the glycerol concentration has to be reduced to biocompatible levels. Freezing and thawing leads to haemolysis of the older more fragile red blood cells. This is turn leads to an increase in the supernatant haemoglobin concentration. Before transfusion the supernatant haemoglobin concentration also has to be reduced to biocompatible levels.

Thawed cells are processed in order to reduce both the glycerol and supernatant haemoglobin concentration. The military processes thawed red blood cells by batch centrifugation using a Haemonetics Cell Washing System 115 (Braintree, MA). The process consists of two steps. After thawing, the glycerol and supernatant haemoglobin concentration are reduced by adding solutions of decreasing osmolality. At the end of this

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predilution step physiological saline can be used for washing without damaging the cells [1]. The entire process, predilution and washing, takes about 45 min and requires 1.5 l of saline.

After predilution, the glycerol concentration is 1.57 M, the supernatant haemoglobin concentration 540 mg dl^{-1} and the haematocrit 15%. After washing the glycerol concentration must be reduced to less than 0.1 M and the supernatant haemoglobin concentration to less than 200 mg dl^{-1}. The haemotocrit must be increased to more than 40%.

Though red blood cells washed with the Haemonetics system meet the required criteria for transfusion there are a number of disadvantages with the procedure. Due to the presence of a rotating seal, the system is not closed to the atmosphere and is thus open to contamination. Consequently the post-thaw shelf life of the blood is only 24 h. In times of war, this leads to many logistical problems as well as wasted units. Further the centrifuge is large (9.1 ft^3), heavy (154 lbs), floor mounted and labour intensive requiring trained operators. These requirements are particularly onerous on the military.

An alternative agglomeration method to remove dimethylsulfoxide (an alternative cryoprotectant to glycerol) from frozen red blood cells has been proposed by Huggins [3]. However, red blood cell recoveries are low, 75–85% compared to over 90% from the Haemonetics Cell Washer. Further this system is also open to the atmosphere and thus possible contamination.

In this study we consider the feasibility of using membrane filtration to achieve a structurally closed system capable of concentrating and washing thawed blood. We shall focus upon the washing step. The predilution step will remain the same as that currently used by the Navy [1,2]. Today membranes are used for many blood processing applications including haemodialysis, haemofiltration, plasmapheresis and cell washing as well as the separation, purification and concentration of blood plasma products. In the deglycerolization process glycerol, haemoglobin and saline solution pass through the membrane while red blood cells are retained by the membrane. Fresh saline is added to the blood bag to replace the permeate removed. Thus continual dilution leads to a decrease in glycerol and haemoglobin concentration.

In the past a few attempts have been made to develop a closed system in order to increase the post-thaw shelf life of washed red blood cells. Zelman and co-workers [4] froze red blood cells in a blood bag made of a nonporous dialysis membrane. The bag was permeable to glycerol but formed an absolute barrier to pyrogens, viruses and bacteria. Thus there was no possibility of contamination from the atmosphere. After thawing dialysate flowed outside the bag. Glycerol from inside the bag passed through the membrane and was removed in the dialysate stream. As haemoglobin could not pass through the nonporous bag it was essential to minimize the amount of haemolysis during washing.

Kleinstreuer and co-workers [5] modelled the system developed by Zelman et al. [4]. Their model predicts the optimum salt concentration as a function of time in the dialysate in order to minimize both the processing time and haemolysis during washing. However, since freezing and thawing also cause haemolysis a major disadvantage of this method is the difficulty in reducing the supernatant haemoglobin concentration during washing. Further the equipment is bulky and difficult to operate in the field during battle.

Radovich [6] has considered the use of commercially available haemodialysers. The membranes used in these modules are also nonporous. Non-porous membranes provide a significant membrane resistance to permeate flow.

In another study Zelman and co-workers [7] have investigated the transport of glycerol by electroosmosis through nonporous ionic membranes. While the use of both AC and DC currents increased the glycerol flux compared to nonporous nonionic membranes it was still lower than the fluxes obtained using microporous membranes. Further the system is complicated requiring an AC or DC current source and would be difficult to operate in the field during battle.

Unlike nonporous membranes microporous membranes have membrane resistances much less than the boundary layer resistance. This leads to much larger permeate fluxes and hence shorter processing times. Further by careful selection of the membrane pore size haemoglobin can also be removed during washing. Since these membranes overcome all of the disadvantages of nonporous membranes we shall only consider microporous membranes.

Some investigators have considered the use of microporous membranes. Van Reis [8] used flat sheet

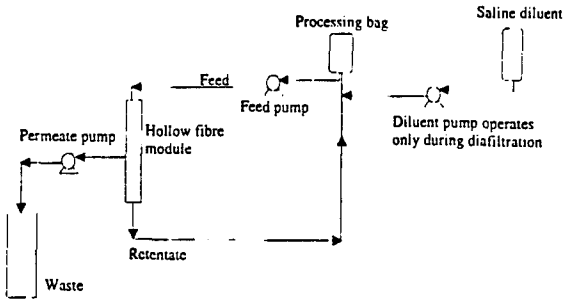

Fig. 1. Schematic representation of the experimental setup. During filtration the diluent pump is turned off and the tubing clamped to prevent retentate flowing into the diluent line.

Nuclepore membranes (Pleasanton, CA). These microporous membranes had an average pore size of 0.6 μm and a thickness of 10 μm. The experimental system used consisted of two pumps; feed and diluent. No permeate pump was used. Friedman and co-workers [9] have considered the use of microporous flat sheet membranes for plasmapheresis. Their experimental system also did not contain a permeate pump.

In this work we shall use microporous hollow fibre membranes in a three pump system; feed, permeate and diluent as shown in Fig. 1. Due to the high porosity and low resistance of microporous membranes initial permeate fluxes are very high. However, these fluxes rapidly decrease as a cake layer forms on the membrane surface. Fane and Chen [10] have shown that these initially high fluxes lead to significant irreversible fouling of the membrane due to haemolysis and subsequent plugging of the pores by red blood cell debris. However, if a permeate pump is used to prevent high initial fluxes until a cake layer develops, less irreversible fouling results and thus higher overall fluxes are obtained.

As well as controlling the initial flux the permeate pump can also be used to control the pressure drop across the membrane during washing. If the pressure drop across the membrane is too high haemolysis of red blood cells and subsequent plugging of the pores by cell debris is likely. By reducing the permeate flow rate the pressure drop across the membrane can be controlled and fouling minimized. This in turn will lead to a higher average flux over the entire washing step and hence shorter processing times.

In a recent study Radovich and co-workers [11] have considered the use of hollow fibres for washing frozen red blood cells. A permeate pump was included in their system. They evaluated the feasibility of using commercially available dialysis, haemofiltration and plasmapheresis modules. As expected they found that plasmapheresis modules which contain microporous fibres gave the highest fluxes and shortest washing times. They determined appropriate operating conditions for removing glycerol using 1200–1750 ml of saline as the diluent fluid. Further they found that their final haemoglobin concentration was less than 150 mg dl^{-1}. However, their results are applicable only to the module and operating conditions used in their study. It is not possible to use their results to predict the performance of a different module with a different membrane surface area and hollow fibres. Further they made no attempt to optimize their system.

To date only van Reis has attempted to optimize the washing process. The analysis he presents is semiquantitative. It is applicable only to his module which used flat sheet membrane since the equation he used to predict permeate flux is empirical and does not account for changes in operating conditions (e.g. wall shear rate) and module dimensions. In this paper we optimize the washing process and present an analysis that can be used for other systems, e.g. removal of cryoprotectants from human platelets, bone marrow cells, etc. [12]. Design equations are developed for specifying the optimum hollow fibre module capable of completing the entire washing process in 30 min and using no more than 1.5 l of diluent. Though we consider the removal of glycerol from frozen red blood cells the analysis we present is generally valid and may be applied to other systems.

2. Theory

During the washing process not only does the glycerol concentration have to be reduced but the blood has to be concentrated. The blood is concentrated by filtration. The glycerol is removed by diafiltration. Thus washing must consist of a combination of filtration and diafiltration steps.

In cross flow microfiltration there is an initial short period of rapid flux decline followed by an extended period of quasi-steady state operation [13,14]. Our deglycerolization process will operate in this region where the mass transfer coefficient is constant. The convection of particles towards the membrane is balanced by their back migration due to Brownian diffusion, shear induced back diffusion and inertial lift. An

increased transmembrane pressure drop leads to an increased driving force for permeation. However it also compresses the deposited layers thus reducing the permeation velocity. Eventually the wall concentration reaches its maximum value at which point the flux attains its maximum pressure independent value.

A number of attempts have been made to model the quasi-steady state region. It was originally thought that the analogy with ultrafiltration of macromolecules would allow the traditional concentration polarization model to predict the steady state microfiltration flux [15,16]. However it has been shown [15,17,18] that for micron sized particles the fluxes predicted using the Brownian diffusivity given by the Stokes–Einstein equation are more than an order of magnitude lower than the observed values. As red blood cells are 2.6 μm in radius (average of major and minor radii), the contribution made by Brownian diffusion to the back migration of the red blood cells may be ignored.

Belfort and co-workers [19–24] have proposed that the lateral migration of particles away from the membrane is due to inertial lift. If the inertial lift velocity is sufficient to off set the opposing permeate velocity particles will not deposit on the membrane. However Belfort et al. [14] show that the inertial lift velocity is dominant for particles greater than 30 μm. Since red blood cells are much smaller it is expected that inertial lift will make a negligible contribution to their back migration.

Zydney and Colton [25] proposed that the concentration polarization model can be applied to microfiltration provided the Brownian diffusion coefficient is replaced by the shear induced hydrodynamic diffusivity first measured by Ecktein and co-workers [26]. They then obtained the following equation:

$$J = 0.078\dot{\gamma}\left(\frac{a^4}{\ell}\right)^{\frac{1}{3}} \ln\left(\frac{H_w}{H}\right) \qquad (1)$$

In Eq. (1) J is the permeate flux, $\dot{\gamma}$ the wall shear rate, a the particle radius, ℓ the active length of the hollow fibre and H_w and H the particle concentrations (or haematocrit in the case of red blood cells) at the wall of the hollow fibre and in the bulk solution respectively. Any consistent set of units may be used.

There are a number of assumptions in the derivation of Eq. (1). Since the maximum wall concentration is used the equation is strictly valid only when the permeate flux is independent of the transmembrane pressure drop. Further the shear induced diffusivity is treated as being independent of particle concentration. Leighton and Acrivos [27] show that the shear induced diffusivity increases monotonically with particle concentration. The derivation of Eq. (1) is based upon the Lévêque solution [28] which assumes a linear velocity profile in the boundary layer. This is only true if the suspension viscosity is a constant which is clearly not the case for red blood cell concentrates [29]. However these latter two assumptions have compensating effects on the permeate flux [30].

The Lévêque solution is only valid for low permeate fluxes. As a result it is valid when the bulk suspension is concentrated [31]. Davis and Sherwood [32] have performed an exact similarity solution for the convective-diffusion equation governing the steady state concentration polarization boundary layer. Their solution includes a concentration dependent effective viscosity and the shear induced hydrodynamic diffusivity reported by Leighton and Acrivos [27]. For washing frozen red blood cells the bulk solution concentration is not dilute so we expect Eq. (1) to give a reasonable estimate of the permeate flux.

The variation of glycerol concentration in the feed bag is easily calculated by constructing a mass balance around the bag. If the rate diluent addition to the bag is equal to the rate of permeate withdrawal and the rate limiting step is convective transport through the hollow fibre membrane, it can be shown that:

$$C(t) = C_0 e^{-V_d(t)/V_b} \qquad (2)$$

where $C(t)$ is the glycerol concentration at time, t, C_0 the initial glycerol concentration, $V_d(t)$ the volume of diluent added after time t and V_b the blood volume permeable to glycerol. The blood volume, must be corrected for the red blood cell volume that is not permeable to glycerol [8]. Thus,

$$V_b = V_b^*\left(1 - \frac{0.3H}{100}\right) \qquad (3)$$

where V_b^* is the actual blood volume and H is the haematocrit. The blood volume and haematocrit at any time during diafiltration, V_b^* and H, are related to the blood volume and haematocrit after predilution, V_0 and H_0 by;

$$V_b^* H = V_0 H_0 \qquad (4)$$

3. Experimental

All experiments were conducted using a Trio Bioprocessing System (Sepracor, Marlborough, MA). The Trio consists of three peristaltic pumps; feed, permeate and diluent. The operator sets the feed and permeate flow rates and the maximum allowable transmembrane pressure drop or transmembrane pressure drop set point. Three pressure transducers measure the inlet, outlet and permeate pressures. During a run if the transmembrane pressure drop exceeds the set point the Trio reduces the permeate flow rate until the transmembrane pressure drop falls below the set point. During diafiltration the diluent pump is a slave to the permeate pump. If the permeate flow is reduced due to a transmembrane pressure drop higher than the set point value the diluent pump is also reduced by the same amount. Thus the permeate and diluent pump speeds are always the same ensuring constant volume diafiltration.

The Trio was programmed to collect data every second. Inlet, outlet and permeate pressures and feed, permeate and diluent flow rates were measured. The Trio calculated the feed and permeate volume processed as well as the transmembrane pressure drop.

A total of 9 hollow fibre modules (Sepracor 300 clarifiers, 430 cm^2 inside surface area containing 163 polysulfone fibres, 14 cm active length, 600 μm i.d., 1000 μm o.d.) were tested. These modules contained fibres with a nominal pore size of 0.22 μm.

Thawed, prediluted blood was provided by the Naval Blood Research Laboratory (Boston, MA). The frozen blood was thawed and prediluted according to the US Navy deglycerolization protocol [1]. Glycerol and haemoglobin concentration were measured by staff of the Naval Blood Research Laboratory. Haematocrit was determined using an International Equipment Company MB Centrifuge (Needham Heights, MA).

A schematic diagram of the experimental system is shown in Fig. 1. Blood is pumped from the feed bag into the lumen side of the hollow fibre module. The retentate is returned to the feed bag. Permeate is pumped into the waste container. During diafiltration the diluent pump replaces the permeate removed with physiological saline.

The experimental system is not closed to the atmosphere since the waste container is not closed. However, the design of a totally closed system is straight forward. The processing bag was made by Dielectric Medistad Company (Boston, MA). It contained two internal baffles to ensure complete mixing of the blood.

4. Results

A total of 16 units of frozen red blood cell concentrate were tested. Four units were used to determine the operating parameters. After predilution the average blood volume was 800 ml with a standard deviation of 86 ml, the haematocrit 15% with a standard deviation of 5.7%, the glycerol concentration 1.57 M with a standard deviation of 0.23 M and the supernatant haemoglobin concentration 540 mg dl^{-1} with a standard deviation of 160 mg dl^{-1}. While these variations are large they are normal for red blood cell concentrates. They represent the large variation between units of donated blood. The washed blood must have a glycerol concentration less than 0.1 M using a maximum of 1.5 l of diluent. From Eqs. (2) and (3) the maximum average blood volume which can be washed using 1.5 l of diluent is 570 ml. An initial filtration step is required therefore, to concentrate the blood before diafiltration.

The higher the feed flow rate the greater the number of passes the red blood cells make through the external circuit and hence the greater the likelihood of haemolysis. In order to minimize haemolysis in the external circuit the blood volume should be recirculated less than once per minute. Thus a feed flow rate of 400 ml min^{-1} was chosen. This corresponds to a wall shear rate of 2000 s^{-1} for our hollow fibre modules.

The maximum transmembrane pressure drop set point was determined to be 80 mmHg. The transmembrane pressure drop is defined as the average of the inlet and outlet pressures minus the permeate pressure. Above 80 mmHg excessive haemolysis was observed at a wall shear rate of 2000 s^{-1}. As the haematocrit of the blood increases, the permeate flow rate will fall after the transmembrane pressure drop reaches the set point value of 80 mmHg.

Diafiltration commenced once the permeate flow fell to 20 ml min^{-1} or the blood volume was about 400 ml. At permeate flows less than 20 ml min^{-1} the washing time becomes unacceptably long. At blood volumes less than 400 ml the blood is recirculated more than once per minute leading to excessive haemolysis in the external circuit. During diafiltration the inlet pressure

and consequently the transmembrane pressure drop decreases with time. This is due to a decrease in the viscosity of the feed solution with decreasing glycerol concentration.

The next 8 units of blood were washed using five hollow fibre modules. Three hollow fibre modules were used to wash two units consecutively. The remaining 4 units were washed using the same hollow fibre module. Washing multiple units with the same module had little effect on the processing time per unit. Thus even after washing 4 units of blood we were still operating in the quasi-steady state region.

Fig. 2 shows the variation of glycerol concentration with diluent volume. The solid line is the result of plotting Eq. (2) the dotted lines represent error bars. We estimate our maximum error in the parameter $V_d(t)/V_b$ to be $\pm 30\%$. Errors in C/C_0 are assumed to be negligible. As can be seen Eq. (2) accurately predicts the removal of glycerol.

Fig. 3 is the analogous plot for haemoglobin removal. Again the solid line is the prediction, the dashed lines represent the error limits. Our prediction is based on Eq. (2) applied to haemoglobin removal rather than glycerol removal. In this case Eq. (2) becomes;

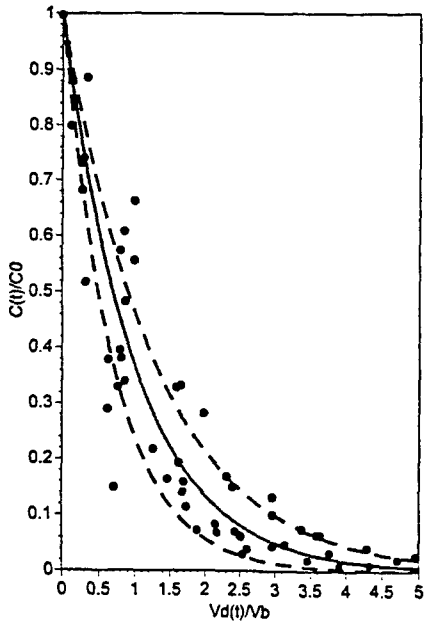

Fig. 2. Variation of glycerol concentration with diluent volume. The solid line represents the predicted glycerol concentration while the dashed lines represent error bars. As can be seen there is close agreement between experiment and theory.

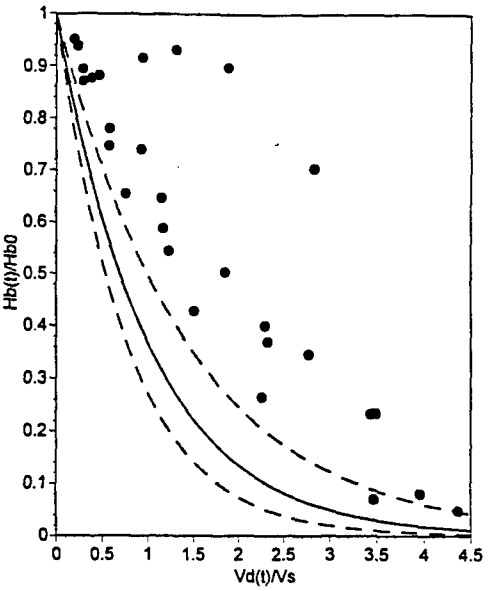

Fig. 3. Variation of haemoglobin concentration with diluent volume. The solid line represents the predicted haemoglobin concentration while the dashed lines represent error bars. As can be seen the actual haemoglobin concentration is higher than the predicted values.

$$\frac{Hb(t)}{Hb_0} = e^{-V_{d(t)}/V_s} \tag{5}$$

where $Hb(t)$ is the supernatant haemoglobin concentration at time t, Hb_0 is the initial supernatant haemoglobin concentration and V_s in the cell free supernatant volume. V_s is related to the actual blood volume by;

$$V_s = V_b^*\left(1 - \frac{H}{100}\right) \tag{6}$$

As before the maximum error in the parameter $V_d(t)/V_s$ is estimated to be $\pm 30\%$. Though not shown errors in Hb/Hb_0 are estimated to be $\pm 6\%$. Unlike Fig. 2, Fig. 3 shows that Eq. (5) does not accurately predict the variation of haemoglobin concentration with diluent volume. The experimentally determined concentration is higher than that predicted.

5. Discussion

An optimized washing process is one that minimizes the overall processing time as well as the diluent volume. Form Eq. (2) it is clear that for a given $C(t)/C_0$ the diluent volume is minimized by minimizing the blood volume. However as the blood volume decreases,

the haematocrit increases hence reducing the permeate flux.

The washing process will consist of two operations, microfiltration and diafiltration. The time required to concentrate the blood from an initial haematocrit of about 15% to the final haematocrit of over 40% will depend on the permeate flux and the membrane surface area. The permeate flux is predicted by Eq. (1). Having determined the wall shear rate, all unknowns are defined in Eq. (1) thus the permeate flux is fixed. For a 300 clarifier the membrane surface area is also fixed. Hence optimization of the washing process involves minimizing the time to complete the diafiltration step.

Substituting for the average radius of a red blood cell (2.6 μm), the active length of our hollow fibre module (14 cm) and the wall concentration into Eq. (1) gives

$$J = 0.0644 \ln\left(\frac{80}{H}\right) \tag{7}$$

where the permeate flux is in cm min^{-1}. A wall concentration of 80% v/v has been assumed. Colton [33] states that the wall concentration can vary from 80–99% v/v. However, since frozen red blood cells are much less flexible than fresh cells they will be less tightly packed. Hence we use the lower end of the range of wall concentrations.

The diluent volume added is a function of time:

$$V_d(t) = JAt \tag{8}$$

where A, the membrane surface area is 430 cm^2 for our modules. By substituting Eqs. (3), (4), (7) and (8) into Eq. (2) we obtain an equation relating the haematocrit at which diafiltration commences to the diafiltration time.

$$\frac{\ln\left(\frac{C_0}{C}\right) V_0 H_0 \left[1 - \left(\frac{0.3H}{100}\right)\right]}{27.7H \times \ln\left(\frac{80}{H}\right)} = t \tag{9}$$

Fig. 4 is a plot of Eq. (9). The solid line is obtained from our average results for C_0, V_0 and H_0. The diafiltration time increases as C_0, H_0 and V_0 increase. This corresponds to a unit with a larger than average volume, haematocrit and glycerol concentration. This limiting case is shown by the upper dashed curve in Fig. 4 which represents the effect on the diafiltration time when C_0, H_0 and V_0 are all three standard deviations greater than

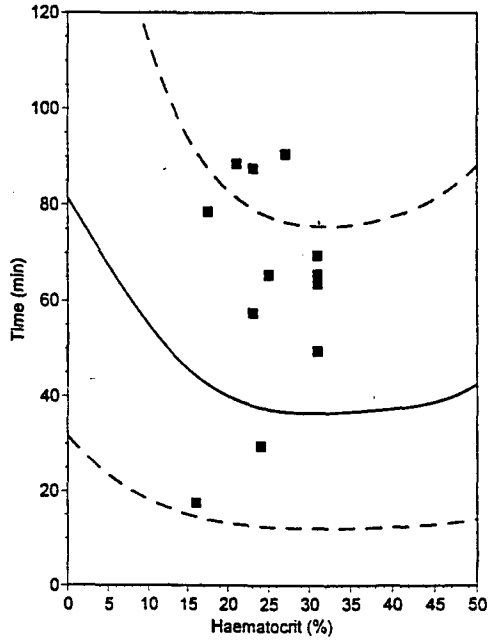

Fig. 4. Variation of diafiltration time with haematocrit. The solid line represents the predicted values. The dashed lines are error bars. Most of the experimentally determined diafiltration times lie within the range of predicted values.

their mean value. The lower dashed curve is for C_0, H_0 and V_0 three standard deviations less than their mean value and represents the opposite limiting case. The wide range between the upper and lower limiting cases is due to the large variation in the initial volume, haematocrit and glycerol concentration of the red blood cells. The experimental data are also included.

Fig. 4 shows that while most of the experimental diafiltration times lie within the range of predicted values a few are higher. This is not surprising since Eq. (1) predicts the pressure independent flux or the maximum quasi-steady state permeate flux. It is not possible to control the transmembrane pressure drop by controlling the permeate flow rate at permeate fluxes that are very close to the pressure independent flux. In reality we always operate below the pressure independent permeate flux.

Eq. (2) is valid for constant volume diafiltration. Though the Trio ensures that the permeate and diluent pump speeds are the same small differences in the outputs of the two pumps are inevitable due to variations in the dimensions of the tubing segments. Over a period of 60 to 90 min this will lead to changes in the blood volume and will affect the diafiltration time.

At both low and high haematocrits the diafiltration time is a strong function of haematocrit. The minimum diafiltration time occurs at a haematocrit between 32 and 33% and is independent of the starting blood volume, haematocrit and glycerol concentration. Near the minimum, the diafiltration time is a weak function of haematocrit. Thus it is not necessary to conduct the diafiltration step exactly at the optimum haematocrit in order to minimize the diafiltration time. Fig. 4 shows that to minimize the diafiltration time the haematocrit of the blood should be between 25 and 35%. However, operating at a higher haematocrit has the advantage of reducing the diluent volume.

As the haematocrit is decreased form the optimum value the blood volume increases as does the diluent volume. However the permeate flux also increases since the feed is less concentrated hence the diafiltration time remains essentially constant. However as the haematocrit is decreased even further the increase in diluent volume dominates and the diafiltration time rapidly increases.

As the haematocrit is increased from the optimum value the permeate flux and the diluent volume decrease. Initially the two effects cancel each other so the diafiltration time does not change. However as the haematocrit is increased further, the decrease in permeate flux dominates hence the diafiltration time begins to increase.

As excepted the minimum diafiltration time increases as C_0, H_0 and V_0 increase. Interestingly as C_0, H_0 and V_0 increase the minimum in the curves shown in Fig. 4 are less shallow. This means the range of haematocrits over which the diafiltration time is close to the minimum value is reduced.

According to the analysis of Ng and co-workers [34] and Cooper and Booth [35] the optimum haematocrit at which to commence diafiltration is H_w/e where e is the natural base of logarithms. Since H_w is 80%, the predicted optimum haematocrit is 29.4%. Eq. (9) on the other hand predicts an optimum haematocrit of 32.5%. The two predictions are close. However in the system considered here, the blood volume permeable to glycerol depends on the haematocrit. The higher the haematocrit the lower the blood volume permeable to glycerol. By increasing the haematocrit the total amount of glycerol present in the blood is reduced as is the time taken to complete diafiltration. Thus our analysis predicts a higher optimum haematocrit. In the analysis presented by Ng et al. and Cooper and Booth the entire feed volume is available to all solutes.

Fig. 4 shows that an optimized washing process requires three steps. First the prediluted blood is concentrated to a haematocrit of 32%. Next diafiltration is conducted. Finally the blood is concentrated to a haematocrit over 40%.

Substituting Eqs. (3) and (4) into (2) we obtain

$$V_d(t) = \ln\left(\frac{C}{C_0}\right) V_0 H_0 \left(\frac{1}{H} - 0.003\right) \tag{10}$$

This equation may be used to predict the diluent volume needed for diafiltration at a given haematocrit and set of initial conditions. Using 1.5 l of diluent and the average values for C_0, H_0 and V_0, Eq. (10) predicts that a haematocrit of 20% is required. Operating at a haematocrit closer to 30% will reduce both the diafiltration time and diluent volume. However, if C_0, H_0 and V_0 are all three standard deviations greater then their average value Eq. (10) predicts a haematocrit of 40% in order to wash the unit with 1.5 l of diluent. The diafiltration time however will be compromised.

During freezing and thawing older red blood cells haemolyse leading to an increase in the supernatant haemoglobin concentration. The average haemoglobin concentration at the start of the diafiltration step was 540 mg dl^{-1} with a standard deviation of 160 mg dl^{-1}. The large variation in the initial supernatant haemoglobin concentration is not unexpected. Before reinfusion the concentration must be below 200 mg dl^{-1}.

Fig. 3 shows that the removal of haemoglobin is not well predicted by Eq. (5). The actual haemoglobin concentration is higher. We believe this is due to rejection of haemoglobin by the membrane in the presence of red blood cells. No rejection of haemoglobin suspended in buffered saline was observed.

We wish to estimate the haemoglobin concentration in the feed bag at any time t during diafiltration. We begin with a supernatant haemoglobin balance around the feed bag.

$$V_s \frac{dHb(t)}{dt} = -Q_p Hb_p \tag{11}$$

where V_s is the cell free supernatant volume, Q_p the permeate flow rate and Hb_p the haemoglobin concentration in the permeate. A supernatant haemoglobin balance around the hollow fibre module yields;

$$Hb(t)Q = Q_p Hb_p + (Q - Q_p)Hb_r \quad (12)$$

where Q is the feed flow rate and Hb_r is the supernatant haemoglobin concentration in the retentate.

The apparent rejection coefficient, σ, is defined as;

$$\sigma = 1 - \frac{Hb_p}{Hb_r} \quad (13)$$

Substituting Eq. (13) into Eq. (12) the haemoglobin concentration in the permeate can be eliminated.

$$Hb_r = \frac{Hb(t)Q}{[Q_p(1-\sigma) + Q - Q_p]} \quad (14)$$

Substituting Eqs. (14) and (13) into Eq. (11) and integrating the resulting expression assuming the permeate flow is much less than the feed flow, i.e. $Q_p/Q \ll 1$,

$$\frac{Hb(t)}{Hb_0} = \exp\left\{-\frac{Q_p(1-\sigma)t}{V_s}\right\} \quad (15)$$

But $Q_p t$ is equal to the diluent volume added. Thus;

$$\frac{Hb(t)}{Hb_0} = \exp\left\{-\frac{(1-\sigma)V_d(t)}{V_s}\right\} \quad (16)$$

A plot of $\ln(Hb(t)/Hb_0)$ versus $V_d(t)/V_s$ should give a straight line with a slope of $-(1-\sigma)$ as shown in Fig. 5. The average rejection coefficient is 0.43. This rejection coefficient is only valid for the operating conditions in this study. Further, it is assumed that the rejection coefficient is constant during the run and that there is no haemoglobin generation due to haemolysis during diafiltration. Our experimental results support these assumptions.

Eq. (16) predicts that for an average unit the supernatant haemoglobin concentration after diafiltration will be 165 mg dl^{-1} which is acceptable. However, for a unit with a higher than average supernatant haemoglobin concentration but average glycerol concentration, the supernatant haemoglobin concentration after diafiltration will be above 200 mg dl^{-1}. For such units the diluent volume required for a given blood volume V_b^*, should be based on Eq. (16) rather than Eq. (2).

The discussion so far has focussed on minimizing the diafiltration time yet ensuring that both the glycerol and supernatant haemoglobin concentrations meet the required specifications. We shall now focus upon the final requirement, that the total processing time (filtration and diafiltration) be under 30 min. Fig. 4 shows that for the hollow fibres modules used in this study the time taken to complete just the diafiltration step is more than 30 min. Thus we need to design a new hollow fibre module.

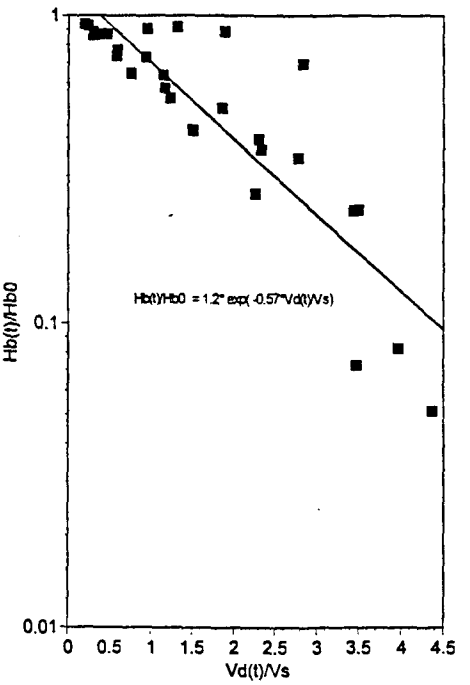

Fig. 5. Variation of the logarithm of haemoglobin concentration with diluent volume. By plotting $\ln(Hb(t)/Hb_0)$ against $V_d(t)/V_s$ the negative of the gradient is equal to $(1-\sigma)$. The solid line is the line of best fit for our experimental data. The apparent rejection coefficient is 0.43.

In the following analysis, it is assumed that the maximum trans-module pressure drop (inlet − outlet pressure) is 100 mmHg. In our experiments the trans-module pressure seldom rose above this value. When it did, the inlet pressure was close to 200 mmHg and a large amount haemolysis was observed. A wall shear rate of 2000 s^{-1} is used.

The viscosity of the blood varies during processing. During the first filtration step the viscosity increases with haematocrit. During diafiltration, the viscosity decreases as the glycerol concentration decreases. Finally during the second filtration step the viscosity increases again. The highest viscosity during diafiltration occurs at the start of the diafiltration step. This was calculated to be 6.9×10^{-3} Pa s. We shall use this value in our calculations.

The feed flow rate through the fibres is given by the Hagen Poiseuille law [36]

$$Q=\frac{\Delta P R^4 n\pi}{8\mu\ell} \tag{17}$$

where Q is the feed flow rate, ΔP the trans-module pressure drop, n the number of fibres, R the inside radius and ℓ the length of the fibres and μ the viscosity of the feed solution. The wall shear rate, $\dot{\gamma}$, is given by:

$$\dot{\gamma}=\frac{4Q}{n\pi R^3} \tag{18}$$

Finally the surface area available for mass transfer, A, is given by:

$$A=2n\pi R\ell \tag{19}$$

The above equations may be solved simultaneously to give Q, n and ℓ as functions of R. The results are as follows:

$$Q=\frac{AR\mu\dot{\gamma}^2}{4\Delta P} \tag{20}$$

$$n=\frac{A\mu\dot{\gamma}}{\Delta P\pi R^2} \tag{21}$$

$$\ell=\frac{\Delta P R}{2\mu\dot{\gamma}} \tag{22}$$

Eqs. (20)–(22) are plotted in Figs. 6-8. Fig. 4 shows that the maximum diafiltration time is 90 min. In order to complete the entire washing process in 30 min we need four times the membrane area since the washing time is directly proportional to membrane surface for a given set of operating conditions. Figs. 6-8 are for a membrane surface area of 1720 cm^2 or four times that present in a 300 clarifier.

Eq. (20) which is plotted in Fig. 6 shows that flow rate varies linearly with fibre diameter. Using fibres with a smaller diameter reduces the recirculation rate which is advantageous. The flow rate also varies linearly with membrane surface area and viscosity. However, it varies with the square of the wall shear rate and inversely with pressure drop.

Eq. (21) which is plotted in Fig. 7, shows that the number of fibres required varies with the inverse square of the fibre diameter. It is also proportional to the viscosity, wall shear rate and membrane surface area but inversely proportional to the pressure drop.

Finally, Eq. (22), plotted in Fig. 8 shows that fibre length varies linearly with fibre diameter and pressure drop. It is inversely proportional to viscosity, a consequence of Eq. (17). The fibre length also varies inversely with wall shear rate.

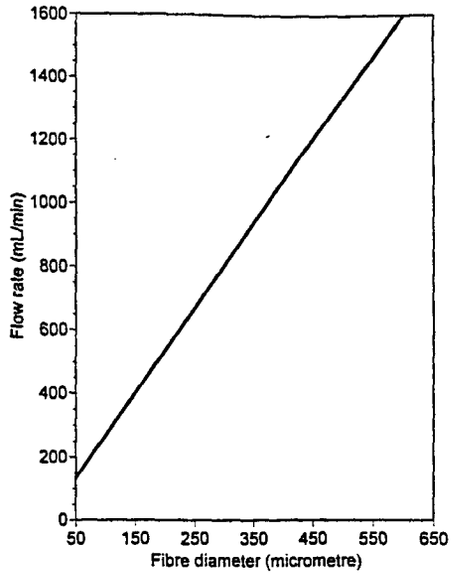

Fig. 6. Variation of flow rate with fibre diameter. As can be seen the feed flow rate varies linearly with fibre diameter. The membrane surface area was 1720 cm^2 and the wall shear rate 2000 s^{-1}. It was further assumed that the trans−module pressure drop was 100 mmHg and the blood viscosity 0.0069 Pa s.

Eqs. (20)–(23) predict the effect of changing a given parameter on the feed flow rate and the number

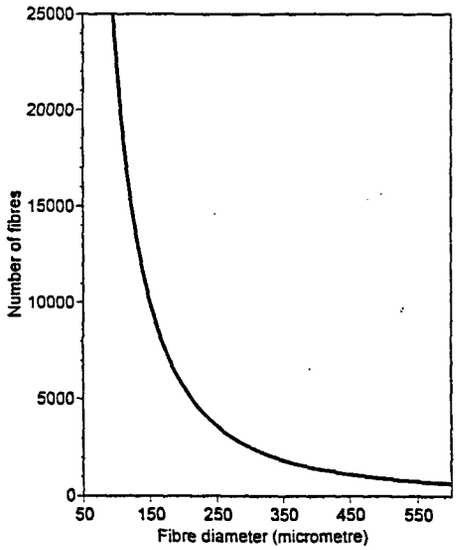

Fig. 7. Variation of the number of fibres with fibre diameter. The number of fibres varies with the inverse square of the fibre diameter. The membrane surface area was 1720 cm^2 and the wall shear rate 2000 s^{-1}. It was further assumed that the trans-module pressure drop was 100 mmHg and the maximum blood viscosity was 0.0069 Pa s.

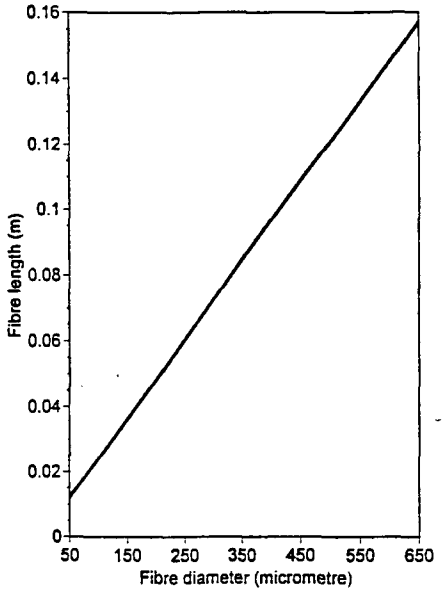

Fig. 8. Variation of fibre length with fibre diameter. As is expected the fibre length varies linearly with fibre diameter. The membrane surface area was 1720 cm^2 and the wall shear rate 2000 s^{-1}. It was further assumed that the trans-module pressure drop was 100 mmHg and the maximum blood viscosity was 0.0069 Pa s.

and length of the fibres. For example, doubling the wall shear rate but maintaining all other parameters the same increases the feed flow rate by four times, doubles the number of fibres but halves the fibre length.

Fig. 6 shows that in order to maintain a feed flow rate under 500 ml min^{-1} and yet ensure a wall shear rate of 2000 s^{-1}, the inside diameter of the fibres should be about 200 μm. Fig. 7 shows that about 6000 fibres will be required. From Fig. 8, the active length of the fibres will be about 5 cm. Assuming an outside diameter of 500 μm and a packaging fraction of 0.5, the fibre bundle diameter will be 5.5 cm.

Using smaller diameter fibres offers the advantage of higher surface area yet low feed flow rates for a given wall shear rate. The minimum fibre diameter is limited by practical considerations such as plugging of the fibres due to cell aggregation.

We have presented a method for sizing hollow fibre modules using the washing of red blood concentrates as an example. However, the method is valid for the design of hollow fibre microfiltration and ultrafiltration systems in general. It consists of first identifying the important design considerations, in this case processing time and diluent volume. Experiments were then conducted to determined the operating envelope. Based on these results we modelled the system in order to optimize the operating conditions. Finally we designed a hollow fibre module capable of meeting the process requirements.

6. List of symbols

A	membrane surface area
a	particle radius
$C(t)$	glycerol concentration at time t
C_0	initial glycerol concentration
H	haematocrit
H_0	initial haematocrit
$Hb(t)$	supernatant haemoglobin concentration at time t
Hb_0	initial supernatant haemoglobin concentration
Hb_p	permeate haemoglobin concentration
Hb_r	retentate supernatant haemoglobin concentration
H_w	wall haematocrit
J	permeate flux
ℓ	fibre length
n	number of fibres
ΔP	trans-module pressure drop
Q	feed flow rate
Q_p	permeate flow rate
R	inside radius of fibre
t	time
V_0	initial blood volume
V_b	blood volume permeable to glycerol
V_b^*	blood volume
$V_d(t)$	diluent volume added at time t
V_s	cell free supernatant volume
$\dot{\gamma}$	wall shear rate
μ	viscosity
σ	apparent rejection coefficient

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APPENDIX 3

Report Prepared By: J. W. McClellan & Associates, Inc.

J. W. McCLELLAN & ASSOCIATES, INC.

MEMORANDUM

DATE: 3-13-96

TO: HemaSure, Inc.
S. Ranil Wickramasinghe, Sr. Research Engineer

FROM: K. E. Bahr

SUBJECT: SBIR Blood Wash Project-March 1996, Final Report
Phase I, 1ST Year-System Design & Prototype Build

This report summarizes the activities and accomplishments during the past year, relative to the design and build of the prototype system and the follow on planning for the second year.

1.0 System Design:

The major challenge became the design integration of a functional disposable tube-set with a practical instrument design. Secondly, process driven changes in requirements resulted in many changes to both the CAD design layouts and to the System Specification. The design and build of two Foamcor product mock-ups greatly facilitated the timely resolution to numerous problems in both Tube-Set and Instrument design. By having developed a System Specification at the out set of the project, we were able to control the concept changes in an orderly fashion through out the design activities. The team had a deliberate common technical focus to deliver a functional prototype, on time.

With the design and the supporting development work underway, it became obvious that the development testing requirements would quickly out strip the capability of the TRIO system. Therefore, the time schedule for the prototype had to be advanced, significantly. Schedules were updated weekly or bi-weekly. We were successful in reducing the project schedule by seven weeks. This was accomplished by: 1.Accelerating the design and redesign efforts; 2.Working closer with suppliers to reduce lead time commitments; 3.Improved team communications and decision making; and, 4.Modifing work schedules for assembly and debug efforts.

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The integration of many TRIO common components and the lack of sterile docking with the diluent bags led to a larger than designed footprint for the instrument. However, the prototype work has pointed out the means of achieving the desired foot print in the next design level.

2.0 Prototype Build:

Prototype drawings were reviewed by the team for release per the Bills of Material. McClellan assisted with the purchasing of the custom and standard components by obtaining quotations, answering technical supplier questions and working out lead time trade offs. The materials were purchased and supplied by HemaSure.

During the assembly and debug of the Prototype problems were identified and quickly corrected. The TRIO power supply proved inadequate and was replaced. Pinch valves were reworked to achieve functionality. Some were still marginal and were replaced in the field. The supplier has since made product design improvements which have corrected the weakness. A few other minor corrections were made in hardware, wiring and labeling.

The prototype has proven worthy of implementing the desired process and is functioning as a process test bed for the evaluation of blood washing. It has also been beneficial in developing process and instrument design refinements for future incorporation.

3.0 Second Year Design Planning:

With the completion of the prototype and the review of the data being generated, we are able to identify several design improvements which are being considered for the next design configuration. The prime design attention will be focused on the following objectives:

- Product Size and Ship Board Requirements
- Integrated Shaker Table Design
- Source Compact Peristaltic Pumps
- Refine Instrument and Tube-Set Interface
- Define Simple Operator Control Panel

A preliminary plan for the design and build of the production prototype system has been prepared and submitted. This plan supports the above system design objectives and it provides for anticipated input from the process development efforts by HemaSure. Foamcor mock ups of the new design will be fabricated for evaluation and operator interface study.

The plan requires starting the preliminary selection of design components and visiting a ship site by mid April, 1996. The prototype will then be built by early November, 1996 and ready for performance testing by the end of December, 1996.

Work has started in researching both the shaker table integration and the new peristaltic pumps. Information has been gathered for specification preparation and design consideration. It may be desirable to both bench test some of these new components and possibly retrofit them into the first year prototype for performance evaluation in parallel with the design effort.

encl. 1. SBIR Blood Wash System Design & Build
Plan for Ph II, Year 2 dated 3/4/96

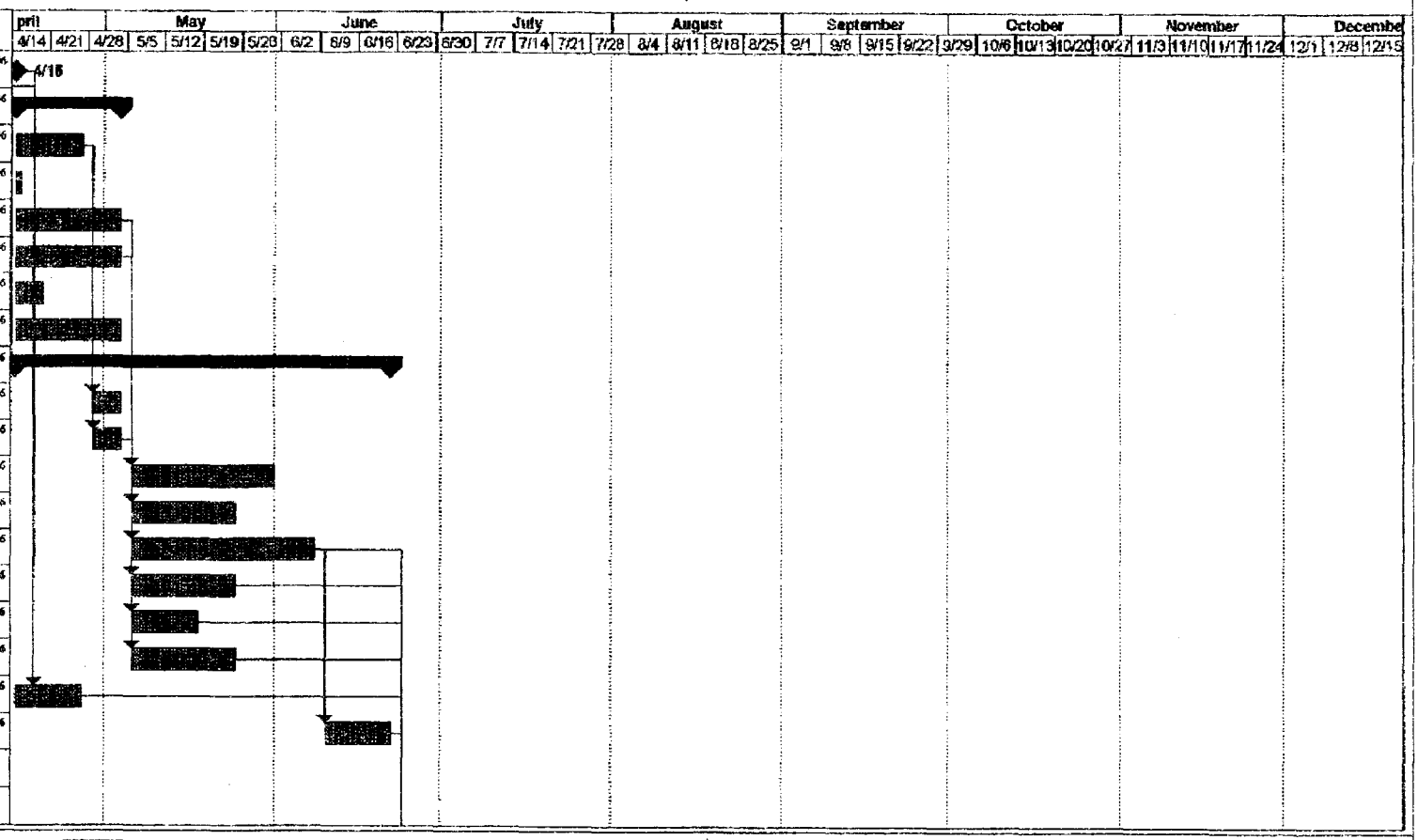
J. W. McCLELLAN & ASSOC., INC.

HEMASURE SBIR PH II - YEAR 2

BLOOD WASH SYSTEM DESIGN & BUILD, 96197HE

3/4/96

ID	Task Name	Duration	Start
1	START PROJECT	0d	4/15/96
2	INFORMATION TRANSFER	15d	4/15/96
3	TEST DATA AVAIL-HE	2w	4/15/96
4	VISIT SHIP INSTALL	1d	4/15/96
5	PUMPS DEFINED	3w	4/15/96
6	SHAKER TBL DEFINED	3w	4/15/96
7	PINCH VLVS CONFIRMD	1w	4/15/96
8	TRANSDUCERS CONFIRMD	3w	4/15/96
9	SPECIFICATION SIGNED	50d	4/15/96
10	TEST DATA REVU	1w	4/29/96
11	PROCESS DEFINED	1w	4/29/96
12	PRELIM PUMP TST COMPL	4w	5/6/96
13	PRELIM SHAKER TST COMP	3w	5/6/96
14	PRELIM SYST LAYOUT	5w	5/6/96
15	PRELIM OPER CONTL LO	3w	5/6/96
16	PO DEFINED	2w	5/6/96
17	DRAFT SPEC	3w	5/6/96
18	DESIGN PLAN	2w	4/15/96
19	PRELIM TUBE SET LO	2w	6/10/96
20			
21			



Project: 96197HE - HEMASURE-YR 2

Task
Progress
Milestone
Summary
Rolled Up Task
Rolled Up Milestone
Rolled Up Progress

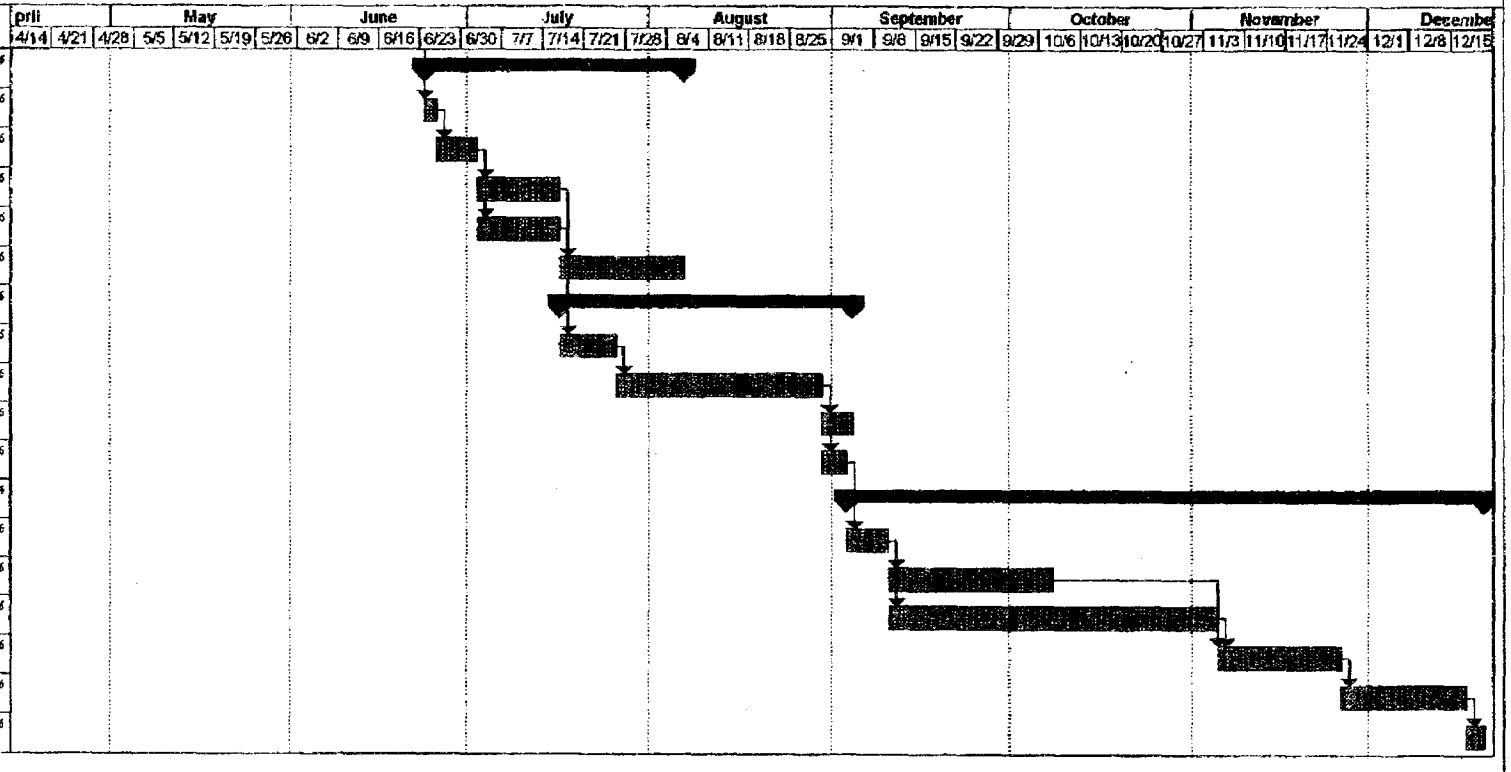
J. W. McCLELLAN & ASSOC., INC.

3/4/96

HEMASURE SBIR PH II - YEAR 2

BLOOD WASH SYSTEM DESIGN & BUILD, 96197HE

ID	Task Name	Duration	Start
22	PRELIM DESIGN REVU	32d	6/24/96
23	REVU ALL LAO, IAO, SPEC	2d	6/24/96
24	UP DATE LAYOUTS	1w	6/26/96
25	TUBE SET AVAIL	2w	7/3/96
26	FOAMCOR MOCK UP	2w	7/3/96
27	OPER INTERFACE EVAL	3w	7/17/96
28	FINAL DESIGN	35.5d	7/17/96
29	UPDATE DESIGN LAO	1.5w	7/17/96
30	PREP DETAIL DWGS	5w	7/26/96
31	PREP BOM	3d	8/30/96
32	DETAIL REVU	2d	8/30/96
33	FAB, ASSEM, DEBUG	73d	9/3/96
34	RELEASE DWGS & POS	1w	9/3/96
35	PURCH COMP AVAIL	4w	9/10/96
36	FAB PARTS AVAIL	8w	9/10/96
37	ASSEMBLE	3w	11/5/96
38	SYST DEBUG	3w	11/26/96
39	SHIP SYSTEM	3d	12/17/96



Project: 96197HE - HEMASURE-YR 2

Task | Milestone | Rolled Up Task | Rolled Up Progress

Progress | Summary | Rolled Up Milestone

Appendix 4

Report Prepared By Ann Harris

Date: Fri, 15 Mar 1996 05:47:17 -0500
X-Sender: rwickram@tiac.net
To: rwickram@hemasure.com
From: rwickram@tiac.net (rwickram)
Subject: 3-96 Summary

>Date: Thu, 14 Mar 1996 22:11:49 -0500
>X-Sender: rwickram@tiac.net
>To: rwickram@hemasure.com
>From: rwickram@tiac.net (rwickram)
>Subject: 3-96 Summary
>
>>From: Callisto1@aol.com
>>Date: Thu, 14 Mar 1996 09:03:28 -0500
>>To: rwickram@tiac.net
>>Subject: 3-96 Summary
>>
>>Navy Blood Washing System
>>Software Development Summary Report for 1995
>>Compiled by Ann Harris for Hemasure
>>March 15. 1996
>>
>>
>>Software development activities for the blood wash project in 1995 have
>>consisted of the following:
>>
>>1. Developing a Specification for the Blood Wash System Software
>>
>>The blood wash prototype system software was based on Hemasure's TRIO
>>instrument clarification software. The clarification process was modified to
>>perform the entire blood wash process, including the loading, predilution,
>>and collection steps. The blood wash prototype includes a simplified version
>>of the TRIO operator interface and diagnostic functions.
>>
>>2. Blood Wash Process Development using the TRIO Instrument
>>
>>In order to have the blood wash software as complete as possible when the
>>prototype was ready for testing, further process development was performed
>>using the existing TRIO instrument. All blood wash process steps were
>>successfully simulated and step parameters (flow rates, volumes, etc.)
>>tentatively selected. This testing took place throughout the

summer of 1995.
>>
>>3. Conversion of Blood Wash Software to run Prototype Instrument
>>
>>During the months of September and October, the blood wash program developed
>>on the Trio instrument was adapted for the new prototype instrument built by
>>McClellan Engineering. The instrument and program were debugged at
>>McClellan's manufacturing facility before shipment to the Naval Blood
>>Research Lab in November.
>>
>>4. Further Process Development on the Prototype Instrument
>>
>>At this time the prototype is being used to wash actual units of blood in
>>order to verify the process. Due to the need to easily modify the process at
>>this stage of testing, parameter editing and intstrument diagnositic
>>functions have been retained from the TRIO software. When this testing phase
>>is complete, the process will be reevaluated and modified for optimization of
>>blood quality, recovery rate, and processing time.
>>
>>
>>
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Appendix 5

Report Prepared By: Jonathan C. Alt

TO: **Franco Castino**

FROM: **Jonathan C. Alt**

SUBJECT: **Deglyc. of Thawed RBC's Disposables Development Report for SBIR Program - Year 1 Ended 3/31/96**

DATE: **March 5, 1996**

Attached is a summary of the first year development of the disposables portion of the deglyc. of thawed RBC's SBIR project.

The report consists of two sections. The first is a brief summary from Marc A. Dee on the development of the hollow fiber module.

The second section is extracted from Kristen Briggs' and Kevin Ranucci's Major Qualifying Report which details the development of the tubeset.

SECTION I

HOLLOW FIBER MODULE DEVELOPMENT

MARC A. DEE

To: JCA
From: MAD
Re: Year 1 module development overview - SBIR
Date: 03/04/96

Fiber development

Fiber screening was carried out on most of the likely candidates using HemaSure model 300 polysulfone housings. Hollow fibers from Akzo, Mitsubishi and HemaSure were evaluated at this scale, as well as a commercially available hollow fiber device from Microgon.

Several variations of the HemaSure hollow fiber family were tested. The best candidate for the deglycerolization process proved to be a developmental hollow fiber made in-house of small bore dimensions. (500 micron OD x 100 micron wall)

This fiber material is difficult to produce, and quite fragile. This has contributed to the low yields we are encountering in the encapsulation operation.

Housing development

For scaleup to actual process conditions, a larger device was required than the model 300 housing used in the fiber screening process. To expedite the process development, it was decided to use the model 1500 housing for the SBIR modules rather than develop a new housing from square one. (The model 1500 housing has the same effective length as the model 300 housing, but at a much greater diameter). Housing availability was limited however, and a molding run was required to obtain housings for scaleup work. The model 1500 module was originally developed for use in the bioseparations market, and the housings were made from polysulfone to withstand high temperature applications (i.e. autoclaving). Because the SBIR project did not require high temperature stability, and due to the high cost and long lead time of the polysulfone material, it was decided to run the tool using SG7 acrylic polymer.

One major concern with the material change was whether the critical dimensions of both the housing and the end caps (i.e. ultrasonic shear weld diameters) would remain in spec. After molding and cooldown, measurements showed the dimensions to be within tolerance. We checked these observations by welding two units and pressure testing to 25 psi.

Secondary operations are required on both the housing and the end cap prior to encapsulation of the hollow fiber. Subsequent annealing, or stress relief, is required after these machining operations.

After initial testing at the model 1500 scale, it was determined that a shorter device was required to overcome a pressure drop problem during the deglycerolization process. This led to further machining operations to produce the housings of proper dimensions, as well as another bonding operation and integrity test. This change also necessitated modifications to several of the fixtures used to produce the modules. (Encapsulation fixtures; cutoff fixtures; weld fixtures).

Encapsulation development

As mentioned above, initial fiber screening was done at the model 300 scale. During this phase, several polyurethane materials were investigated to encapsulate the various hollow fiber candidates. From these materials, one system was chosen which appeared to suit our needs. (Medical application; low initial viscosity; adequate working life, etc.)

Initial work was done attempting to static pot the fibers, but was abandoned due to inconsistent results. (Wicking of the encapsulant up the hollow fiber was uncontrolled; entrapped air in the encapsulant was a problem; static mixing of the two components proved difficult).

Most of our experience in module fabrication utilized a centrifugal encapsulation process. Existing fixturing used the centrifugal force to move the encapsulant from a reservoir to the molds. Prior experience with high porosity, small bore hollow fibers showed that it was difficult to reach encapsulation conditions where the force was high enough to dispense all of the material to the mold, yet low enough to

prevent the encapsulant from penetrating through the walls of the fiber and plugging up the lumens of the fiber.

This led to the de-coupling of the dispense system from the encapsulation fixturing. (i.e. not relying on the centrifugal forces to dispense the encapsulant). By using a pneumatic injection system, the RPM of the centrifuge could be throttled down to an absolute minimum to prevent plugging of the fiber lumens. Even with these changes, the fiber material of choice could not be encapsulated at optimum packing density, and the fiber quantity had to be adjusted down.

Encapsulation conditions required further adjustments when scaling up to the larger device. After several experiments, the parameters of degas time, injection pressure, max RPM and total spin times were established.

At this time, a new lot of the polyurethane material was received and behaved much differently than the sample lot which we used to nail down the encapsulation parameters. The vendor, had made a formulation change to the material, whhich resulted in a much faster cure rate. To date, we are still struggling to compensate for this formulation change by modifying the polyurethane encapsulation procedure, and production yields are down.

Downstream processing development

After encapsulation, the hollow fiber modules require further processing. These steps include cutoff; inspect; repair (if necessary); wet-out; integrity test; repair (if necessary); cap and dry.

The soft, ductile properties of the polyurethane after initial curing required the development of a new method of cutting the cured polyurethane to expose the lumens of the fibers. A guillotine was retrofitted for this purpose. The resulting cut is clean, but not perfectly straight. A double gasket was added to prevent leakage between the cap and the polyurethane face. (A leak at this seal results in trapped RBC only, not RBC leaking to the outside of the closed system).

The remainder of the downstream processing is an iterative process to ensure that there are no integrity breaches in the hollow fiber. (i.e. no crosstalk of shell flow to lumen flow). The modules are inspected for any gross problems (plugged fibers or encapsulation voids), wetted out with DI water and integrity tested with helium. The amount of work involved is a function of the starting quality of the hollow fiber bundle. Because the fiber is a new developmental fiber, some problems have been encountered at this stage of the process, which have also impacted the production yields.

SECTION II

TUBESET DEVELOPMENT

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4.0 PROCEDURE

The development of the disposable tubeset was carried out in 4 major sets: general design of the tubeset, production of the tubeset, testing of the tubeset, and packaging the tubeset.

GENERAL DESIGN

4.1 TUBING LENGTH DETERMINATION

To ensure a system was created that would allow the tubeset to be easily placed on the hardware, a "mock-up" model of the actual hardware was utilized. The company which designed the hardware system, McClellan Integrated Research of Hollice, NH, constructed the Styrofoam "mock-up." This mock-up provided a model used to determine specific design criteria such as lengths and volumes of tubing. The mock-up model had all the appropriate external features, such as pumps and pinch valves, in the proper locations. This model was integral in the development of the tubeset and ensured that the tubeset and hardware would be compatible.

The initial tubeset was designed with the hardware and washing process steps as key criteria. Predilution, filtration, and concentration of the blood were the three basic phases of the blood processing procedure. The tubeset was designed such that each of the three processing phases were handled by its own tubing section. For example, the processing tubing segments were in a

separate section on the prototype hardware model. The processing steps in separate sections was a design decision that allowed tubing volumes within process segments to be minimal.

4.2 TUBING VOLUME DETERMINATION

One of the criteria for the system was that it was equal to or better than the current systems. The current systems have a final hold - up volume or volume left in the tubeset at the end of the processing. The current system has a hold - up volume of 50 ml. This maximum hold - up volume was a factor in choosing tubing. The total tubing volume within the tubeset is 6.1 ml with 1.6 ml due to 4.1 mm tubing and 4.5 ml due to 6.8 mm tubing. The volume was calculated using the formula Volume = $\pi r^2 l$, where r is equal to the tubing radius and l is the length of the tubing. The tubeset volume was also a critical value because it was a closed system. The volume in each line flowing into a bag had to be calculated to ensure the bag could hold the initial displaced air from the tubing and the fluid. Once these volumes were calculated, the bags and tubeset were designed to accommodate the total volume.

4.3 COMPONENT SELECTION PROCEDURES

Components for the disposable tubeset were selected based on the

following criteria:

- **Gamma Stability**
- **Bonding ability**
- **Biocompatibility**
- **Flow Rate of System**
- **Cost**

The criterion of *Gamma stability* was chosen for component selection because the entire disposable tubeset interfaces with blood and must be a sterile product. As a method of sterilization, gamma irradiation was used and the components were required to maintain integrity at an irradiation rate of 2.5 megarads.

The criterion of *Solvent bonding* was chosen for component selection because the tubeset had to be a "closed" sterile system. The entire tubeset had to be adhered using cyclohexanone. Thus, all components had to be compatible to solvent bonding to complete the "closed" sterile tubeset..

The criterion of *Biocompatibility* was chosen for component selection because tubeset has direct interfacing with blood and blood products. This issue was critical when the selection process was taking place. In order to reduce hemolysis effects throughout the set, proper material selection is required.

The criterion of the *Flow Rates of the system* were chosen for component selection because the cell washing system operates on a pump driven basis. The components had to be compatible with the pump rates and could not limit flow distributed by the pumps.

The criterion of *Cost* was chosen for component selection because it was a limiting factor when selecting components. The SBIR grant allotted a strict budget to HemaSure. HemaSure's goal was to keep the tubeset cost, which includes labor and material, to a value that was competitive with the current systems.

4.3.1 Transition Components

Transition components were utilized to join separate pieces of the tubeset together. Basic Y and T joints made of semi-rigid PVC were utilized for this application. Various diameter sizes of the joint ports, inner diameters of 4.1 mm and 6.8 mm, enabled different sizes of tubing to be joined.

The criteria of solvent bonding, gamma stability and biocompatibility were all satisfied through the material selection of PVC. This is a very common material used in the medical field and in the area of red cell preservation.

4.3.2 Tubing Components

PVC tubing of various inner and outer diameters was utilized in the construction of the tubeset. In tubing selection, two standard classifications of "small" and "large" were determined based on size.

Small tubing, which had an inner diameter of 2.5 mm and a 4.1 mm outer diameter, was selected for the lines that required flow rates of less than 100 ml/min. The flow rate of 100 ml was established by HemaSure criteria for cell processing. The size of this tubing was set by HemaSure and was necessary for sterile docking. Sterile docking is a procedure that joins one tubing to another through a sterile melting technique. This procedure was how the blood supply bags were going to be attatched to the the tubeset and therefore, the tubing had to be the same size as the blood supply bag tubing.

Large tubing, which had an inner diameter of 5.2 mm and 6.8 mm outer diameter was used for the main process loop. This size tubing was selected based on its compatibility with transition pieces (such as Y and T joints) and ability to handle required flow rates of 200 ml/min.

The criteria of solvent bonding, gamma stability and biocompatibilty were all satisfied through the material selection of PVC. More importantly, the criterion of flow rate was satisfied through the selection of specific outer and inner diameter sizes of the small and large tubing. The criterion of cost was also satisfied through the selection of tubing type.

The small tubing was the same tubing used in another product developed by HemaSure and was purchased at a cheaper rate per foot price due to the large quantity purchased.

4.3.3 Sterilization Filters

Sterilizing filters were a necessary component to the tubeset. The filters were placed in tubing lines where a spike was existed to access a washing or predilution solution. The use of the filter ensured sterility of the solution and was a vital component in keeping the system "closed" or sterile. The housing was an injection molded modified acrylic and the membrane was a supor .22 μm membrane suitable for high flow.

The criteria of gamma sterilization and biocompatability were satisfied through the material selection of acrylic. The criterion of flow rate was satisfied through the selection of a high flow filter. Unfortunately, some trade-offs had to occur due to the vital nature of this component. The criterion of solvent bonding was not satisfied through material selection. Instead, ultraviolet light welding (UV) was utilized to attach filters to tubing. Also, the criterion of cost was a trade-off in the selection of this component. It was necessary to pay more for the higher flow rate capability.

4.3.3-1 Testing: Sterilization Filters

Flow Testing:

Sterilization filter flow testing was necessary to establish that flow to the peristaltic pumps would not be slowed due to the filter. The project team tested the filters by simulating the environment of the tubeset. Diluents and salt solutions that would be used in the processing of the blood were attatched to the tubing using spikes. Tubing which led into the filters was run through a peristaltic pump. Flow rates were measured with and without the filter in the tubing line. The highest flow rate in the system on this assembly was 100 ml/min. The supor membrane was chosen because it supplied this flow rate.

Gamma Testing:

Gamma stability was confirmed by irradiating ten samples and passing fluids through them using the pump. Performance level did not decline and the samples were considered gamma stable.

4.2.4 Microaggregate Filters

A microaggregate filter processes thawed blood and removes clots found in the blood. Thawed blood has particlulates that could foul the hollow fiber membrane. A microaggregate filter was necessary in the line of the blood input. A filter that was capable of passing up to 6 units of blood and pumping through the filter using a peristaltic pump was chosen for the tubeset. The inlet port of the filter was ABS and the housing and outlet port was polycarbonate. The filter membrane was a 40 μm membrane suitable for uses in high flow rate requirements.

The criteria of gamma stability and biocompatability were satisfied through material selection of ABS and polycarbonate. The criterion of flow rate was satisfied by the selection of a 40 μm membrane for fast flow. Unfortunately, only half of the criterion for solvent bonding was satisfied through material selection for the component. The techniques of UV welding were needed to bond the ABS part of the filter. Also, the criterion of cost was not satisfied due to the price of the filter. This was a trade-off which was needed due to the necessity and importance of the component.

4.3.4-1 Testing: Microaggregate Filter

Gamma Testing:

Testing for the microaggregate filter was necessary to ensure gamma stability. The filters were tested by the manufacturer for gas stability only. A sample of ten filters were gamma irradiated at 2.5 mega rads. The filters were used to process units of blood and then compared with the performance of the non-irradiated filters. The performance between the non-irradiated and irradiated filters was the same and met the needs of the system. The filters were then deemed gamma stable.

4.3.5 Pressure Transducer Protector

Pressure transducers were used in the hardware to monitor trans-membrane pressure in the module. Pressure transducer protectors were incorporated into the tubeset to protect the hardware from fluid contact. The housing was made of ektar for gamma stability.

The criteria of gamma stability, solvent bonding and biocompatabilty were satisfied by material selection of ektar. The cost criterion was satisfied because there was no other less-expensive component on the market.

4.3.6 Tubeset Bag System

4.3.6-1 Process Bag

The process bag made of PVC was a baffled design capable of recirculating a minimum volume of 100 ml. The baffles also provided a "lag" time period upon which the red cells in the pocket area could allow the cells of higher concentrations of glycerol to rise to the top of thebaffle and spill over for washing. The process bag had three ports: Two outlet poets on either sides of the baffle, and an inlet port in the center of the baffle portion of the bag. The outlet ports were compatible with the 6.8 mm outer diameter tubing.

4.3.6-2 Waste Bag

A 5 liter waste bag was necessary to hold the waste (cryoprotectant, free hemoglobin, and washing solutions) from the washing process. The bag was designed to have one port and 48" of tubing attached. The tubing was the small 4.1 mm outer diameter tubing used throughout the tubeset.

4.3.6-3 Blood Collection Bag

The blood collect bags were standard off the shelf 600 ml blood bags made of PVC. The bags have a single port for tubing and two spike ports.

A complete listing of the components utilized in the development of the tubeset is described in Appendix C.

4.4 MODULE

Membrane diafiltration was utilized to create a structurally closed system capable of concentrating and washing thawed blood. Microporous hollow fiber membranes enclosed in a module were chosen by HemaSure Inc. to perform the deglycerolization. These modules were supplied by HemaSure and incorporated into the design of the tubeset to create an entirely closed and sterile system.

4.5 DEVELOPMENT OF BAG DESIGN

The original process bag baffle was previously designed by HemaSure to handle a minimal recirculating volume of 400 ml (275 ml center volume and 125 ml side volume). This design was taken and readjusted to meet the system specification of 100 ml (50 ml center volume and 50 ml side volume). This process involved reducing the height of the baffles to allow for the respective volumes. In order to determine the new height, two methods were utilized: an experimental method and a theoretical/mathematical method.

4.5.1 Experimental Method

The experimental method entailed filling the bag with liquid, in this case water, and observing flow characteristics and volumes. In a preliminary experiment, measurements using the existing bag and metal rods to deform the active area of the process bag were attempted. It was difficult to make dimensional predictions for the smaller bag based on the original bag. The experimental based design originated from placing the desired volume, 50 ml, in the original bag's center and marking the level the liquid reached. The baffles were then simply scaled down to the height of the 50 ml. Tracing paper was used to maintain the shape of the original baffles. This experimental method provided a height of the baffles to be 2.5 inches.

4.5.2 Theoretical/Mathematical

In order to determine the height of the new baffle, the theory of simple geometry was used. The first step in calculating the new baffle height for the target volume of 200 ml was to select specific geometry's which would represent the volume in the baffle pocket. The shape of the baffle was modeled as both a cylinder and as 2 cones joined at their bases. The cylinder model represented the upper specification while the two cones represented the lower specification limit.

Cylinder Model: Upper Specification Limit

Volume(Cylinder) = Π(radius)^2 (height) = target volume

where: r= unknown we are looking for

h= 1.56 inches (the thickness of the bag at the baffle pocket)

target volume= 200ml for (inner and side pockets, and crease vol.)

r = 4 inches upper specification limit

Two Cone Model: Lower Specification Limit

Volume(Cones) = 2[(1/3)Π(radius)^2 (height)] = target volume

where: r= unknown we are looking for

h= 1.56 inches (the thickness of the bag at the baffle pocket)

target volume= 200ml for (inner and side pockets, and crease vol.)

r = 3.08 inches lower specification limit

The final design of the process baffles, based upon the theoretical results, was evaluated to have a baffle height in between the specification limits of 3.08" and 4". Because the two cone method was a better representation of the baffle pocket, the baffle height volume was skewed toward the lower specification limit. The baffle height determined from this analysis was estimated at approximately 3.3".

The values of 2.5" and 3.25" were chosen for the new baffle heights. The 3.25" was determined through the analyses. The 2.5" baffle height was chosen through an estimation that the 3.25" would not be low enough.

PRODUCTION

4.6 BONDING PROCEDURES

4.6.1 Solvent Bonding Methods

The solvent bonding technique was performed based on a protocol set forth by Hemasure, Inc. For all processes utilizing solvent bonding for adhesion, the following was considered and performed.

Safety Concerns:

Solvent bonding should be carried out under a hood and safety glasses and rubber gloves should be used at all times.

Materials:

Cyclohexanone
Pipette
Tubing
Fitting

Procedure:

1.) Use the pipette to draw up cyclohexanone.

2.) Hold tubing at one end and use the pipette to dispense the cyclohexanone over the opposite end, covering approximately 1/4" of tubing.

3.) Place the end of tubing with cyclohexanone firmly into the fitting. Visually inspect the bond for air bubbles. If bubbles appear, use the pipette to dispense a small amount of the cyclohexanone directly to the bond site.

4.) Allow to let dry at least 3 minutes before performing the next operation. For complete drying, it is necessary to let the bonds dry for 24 hours.

4.6.2 UV Welding

The UV welding technique was also performed based on a protocol set forth by Hemasure, Inc. For all processes utilizing UV bonding for adhesion, the following was considered and performed.

Safety Concerns:

Protective eyewear that is UV ray protectant should be worn at all times. Rubber gloves are also necessary.

Materials:

Dymax UV Light source
UV adhesive, Dymax 181 - M
UV adhesive, Dymax 190 - M
Tubing
Fitting

Procedure:

Procedure for attaching Spikes and Microaggregate filter:

1.) Place a bead of Dymax UV adhesive 181 - M on the end of the tubing.

2.) Insert the tubing into the end of the spike or microaggregate filter.

3.) Cure the UV for 20 seconds by applying the light source directly down onto the bond site. It is important to do this because the material is ABS and is opaque. The light must be able to reach the bond site.

Procedure for attaching tubing to module and Sterilization filters:

1.) Place a bead of Dymax UV adhesive 190 - M on the end of the tubing. For the Sterilization filter, place a bead onto the inlet and outlet ports of the filter. Slide the tubing over the respective port.

2.) Insert the tubing into the end of the module.

3.) Cure the UV for 20 seconds by applying the light source to the bond site.

4.7 PRODUCTION PROCEDURES

In order to produce the entire disposable tubeset, subassemblies were created and then bonded to together.

4.7.1 Sub-Assembly 1

Materials:

Qty.		Description
3	1D	Spike with cap
2	1A	Y fitting
1	1B	Sterilization filter
2	1C	Blood bag clip
	Tubing	
1	A1	
1	A2	
1	A3	
1	A4	
1	A5	
1	A6	

Procedure:

For a graphic of this subassembly, refer to *Figure 5.1: Sub - Assembly 1,* in the results section .

1. Attach spikes to tubing A1, A2, and A3 using UV welding.
2. Put blood bag clips (1C) onto A1 and A2.
3. Attach A1 into the left side of the Y - (1A) using solvent bonding.
4. Attach A2 into the right side of the Y - (1A) using solvent bonding.
5. Attach A4 into the bottom of the Y - (1A) using solvent bonding.
6. Attach A4 into the left side of the Y - (1A) using solvent bonding.

7. Attach A3 into the right side of the Y - (1A) using solvent bonding.

8. Attach A5 into the bottom of the Y - (1A) using solvent bonding.

9. Attach A5 to the top port of the Sterilization filter (1B) using UV welding.

10. Attach A6 to the bottom port of the Sterilization filter (1B) using UV welding.

11. Attach A6 to sub - assembly 2 per diagram using solvent bonding.

4.7.2 Sub - Assembly 2

Materials:

Qty.		Description
1		Process bag
3	4C	Transition tubing
1	2B	Y fitting
4	2C	T fitting
	Tubing	
1	B1	
1	B2	
1	B3	
1	B4	
1	B5	
1	B6	

Procedure:

For a graphic of this subassembly, refer to *Figure 5.2: Sub - Assembly 2*, in the results section.

1. Attach transition (4C) to the three ports of the Process bag using solvent bonding.

2. Attach B1 to the left port of the process bag using solvent bonding.

3. Attach B2 to the right port of the process bag using solvent bonding.

4. Attach B3 to the middle port of the process bag using solvent bonding.

5. Attach B1 into the left side of the Y - (2B) using solvent bonding.

6. Attach B2 into the right side of the Y - (2B) using solvent bonding.

7. Attach B4 into the bottom of the Y - (2B) using solvent bonding.

8. Lying the Process bag flat, one T - (2C) should be attached to B3 with the 4.1 mm port to the right using solvent bonding.

9. Lying the Process bag flat, one T - (2C) should be attached to B4 with the 4.1 mm port to the left using solvent bonding.

10. Tubing B6 should be attached to the 4.1 mm ports of B3 and B4 using solvent bonding.

11. Attach B5 to open ends of T's (2C) on B3 and B4 using solvent bonding.

12. Attach T's - (2C) to B5, with 4.1 mm port, orientated in the opposite direction of the other T's using solvent bonding.

13. Attach the T - (2C) on the B4 tubing line to B7 using solvent bonding.

14. Attach the 4.1 mm port of the T on the B4 line to sub assembly 3 using solvent bonding.

15. Attach the T - (2C) on the B3 tubing line to sub assembly 6 using solvent bonding.

16. Attach the 4.1 mm port of the T on the B3 line to sub assembly 1 using solvent bonding.

4.7.3 Sub Assembly 3

Materials:

Qty.		Description
1	1D	Spike
2	1C	Blood bag clip
1	1B	Sterilization Filter
1	3A	Three port Y
2	3B	Transition tubing
1	3C	Microaggregate filter
	Tubing	
1	C1	
1	C2	
1	C3	
1	C4	
1	C5	
1	C6	

Procedure:

For a graphic of this subassembly, refer to *Figure 5.3: Sub - Assembly 3,* i in the results section .

1. Attach spike (1D) to tubing C2 using UV welding.
2. Slide blood bag clamp (1C) over tubing C2.
3. Attach C2 to inlet port of Sterilization filter (1B) using UV welding.
4. Attach C3 to the outlet port of the Sterilization filter (1B) using UV welding.
5. Attach C1 to the left port of the three port Y - (3A) using solvent bonding.
6. Attach C3 to the center port of the three port Y - (3A) using solvent bonding.
7. Attach C4 to the right port of the three port Y - (3A) using solvent bonding.

8. Attach C5 to the bottom port of the three port Y - (3A) using solvent bonding.

9. Attach tubing transition (3B) to C5 using solvent bonding.

10. Attach tubing transition (3B) to the top port of the microaggregate filter (3C) using UV welding.

11. Attach tubing transition (3B) to the bottom port of the microaggregate filter (3C) using solvent bonding.

12. Attach C6 to tubing transition (3B) using solvent bonding.

13. Slide blood bag clamp (1C) over C6.

14. Attach C6 to sub - assembly 2 using solvent bonding.

4.7.4 Sub - Assembly 4

Materials:

Qty.		Description
1	1A	Y fitting
1	2C	T fitting
2	1C	Blood bag clips
2		Blood bags
	Tubing	
1	E1	

Procedure:

For a graphic of this subassembly, refer to *Figure 5.4: Sub - Assembly 4*, in the results section .

1. Attach E1 to the 4.1 mm port on the T - (2C) using solvent bonding.

2. Attach E1 to the bottom port of the Y - (1A) using solvent bonding.

3. Place the 1C blood bag clip onto the blood bag tubing.

4. Attach one blood bag to the right port of the Y - (1A) and one bag to the left port of the Y - (1A) using solvent bonding.

5. Attach the left port of the T - (2C) to B7 using solvent bonding.

6. Attach the right port of the T - (2C) to D9 using solvent bonding.

4.7.5 Sub - Assembly 5

Materials:

Qty.		Description
1		5 Liter Waste Bag
3	3B	Transition tubing
3	B5	Transition tubing
1	2C	T fitting
1	4D	Pressure transducer
1	4E	Luer fitting
	Tubing	
1	D5	
1	D10	

Procedure:

For a graphic of this subassembly, refer to *Figure 5.5: Sub - Assembly 5,* in the results section.

1. Attach the end of the waste bag to a 3B transition using solvent bonding.

2. Attach the 3B transition to a B5 transition using solvent bonding.

3. Attach the B5 transition to the left port of T - (2C) using solvent bonding.

4. Attach the curled D5 tubing to the 4.1 mm port of the T - (2C) using solvent bonding.

5. Attach tubing D5 to the male luer fitting on the pressure transducer (4D) using UV Welding.

6. Attach the luer fitting (4E) to the female luer fitting on the pressure transducer (4D) using UV Welding.

7. Attach tubing D10 to the luer via the tubing port using solvent bonding.

8. Attach the B5 transition to the right port of the T - (2C) using solvent bonding.

9. Attach the 3B transition to the B5 transition using solvent bonding.

10. Attach tubing D5 to transition 3B using solvent bonding.

11. Attach other end of tubing D5 to transition 3B using solvent bonding.

12. Attach transition 3B to transition B5 using solvent bonding.

13. Attach to sub - assembly 6 per diagram using UV welding.

4.7.6 Sub - Assembly 6

Materials:

Qty.		Description
1		Hollow fiber module
2	4A	Transition tubing
1	4B	Module transition
2	4C	Transition tubing
2	B5	Transition tubing
2	2C	T fitting
2	4D	Pressure transducer
2	4E	Luer fitting
	Tubing	
1	D1	
1	D2	
1	D3	
1	D7	
1	D8	
1	D9	

Procedure:

For a graphic of this subassembly, refer to *Figure 5.6: Sub - Assembly 6*, in the results section.

1. Attach tubing transition (4A) to the top port of the module using UV welding.
2. Attach tubing transition (4C) to tubing transition (4A) using solvent bonding.
3. Attach tubing transition (4A) to the left (bottom) port of T - (2C) using solvent bonding.
4. Attach the curled D2 tubing to the 4.1 mm port of the T - (2C) using solvent bonding.
5. Attach tubing D2 to the male luer fitting on the pressure transducer (4D) using UV welding.
6. Attach the luer fitting (4E) to the female luer fitting on the pressure transducer (4D) using UV welding.
7. Attach tubing D3 to the luer via the tubing port using solvent bonding.
8. Attach D1 to right (top) port of T - (2C) using solvent bonding.
9. Attach D1 to sub - assembly 2 using solvent bonding.
10. Attach sub - assembly 5 to module transition (4B) using UV welding.
11. Attach tubing transition (4A) to the bottom port of the module using UV welding.
12. Attach tubing transition (4C) to tubing transition (4A) using solvent bonding.
13. Attach tubing transition (4A) to the right (top) port of T - (2C) using solvent bonding.
14. Attach the curled D7 tubing to the 4.1 mm port of the T - (2C) using solvent bonding.

15. Attach tubing D7 to the male luer fitting on the pressure transducer (4D) using UV welding.

16. Attach the luer fitting (4E) to the female luer fitting on the pressure transducer (4D) using UV welding.

17. Attach tubing D8 to the luer via the tubing port using solvent bonding.

18. Attach D9 to left (bottom) port of T - (2C) using solvent bonding.

19. Attach D9 to sub - assembly 4 using solvent bonding.

TESTING

4.8 INTEGRITY TESTING PROCEDURES

Integrity testing was performed on the subassemblies and all bond areas to ensure the disposable tubeset was a "closed" sterile system. This testing was performed in three different ways: visual, pull, and pressure decay.

4.8.1 Visual Testing

Visual testing was performed on all solvent bonded and UV bonded areas. In order for a bond area to pass inspection, there had to be a clear bonding area present. No bubbles in the bond were allowed. This inspection was performed on all of the solvent bond and UV bonds.

4.8.2 Pull Testing

Pull testing methods were performed on all solvent bonded and UV bonded areas. After each bond was set, the respective components on both sides of the bond area were pulled by hand in tension at approximately 5 lb. force. Testing was performed on all bonded areas of the disposable tubeset.

4.8.3 Pressure Decay

Pressure Decay testing methods utilized the incompressible property characteristics of air. Compressed air, regulated by a pressure regulator, was discharged into each respective subassembly. The open ends of the assembly, except the area where the air was entering, were closed off with hemostats. The subassembly was then pressurized to 5psi and held there for approximately 30 seconds. If there was a bond leak, the pressure would decay and the subassembly would be rejected.

PACKAGING

4.9 Tubeset Packaging Procedures

Packaging of the tubeset must be done to ensure no kinking of tubing occurs, the set is easy to place on the machine and is ready for gamma irradiation. The tubing used was coiled in its raw material form, and therefore

had a tendency to curl in certain directions. This was taken advantage of when packaging the tubeset. The blood collect bags, for instance, came folded in thirds with tubing wrapped inside. The collect bags are packaged in thirds with the two bags together with an elastic band in the set. The waste bag was manufactured with 48 inches of tubing attached. The bags were shipped folded in half with the tubing coiled in the middle. The waste line was packaged with the same principle. The tubeset was divided into three sections for packaging. The diluent line (sub - assembly 1), which is on the left, is coiled loosely and placed on top of the bypass line (B6) in the center. The blood input line (sub - assembly 3), which is on the right, is coiled loosely and placed on top of the diluent line. The process bag is folded over this tubing assembly and the tubeset is placed into a ziploc storage bag . The waste line is placed in the bag on top of this assembly and the storage bag is sealed.

5.0 RESULTS

INITIAL DESIGN RESULTS

The process bag was tested at HemaSure, Inc, using water to ensure that the function of baffle design was successful. The two designs of the bag were found to recirculate the minimum volume and the design was satisfactory. The bag with baffles at the height of 2.5 inches was chosen for the tubeset because it required a lower volume for recirculation.

The initial procedures were only carried out for the hardware/tubeset interface. The methods of integrity testing and production were not performed until the actual design was final.

FINAL DESIGN RESULTS

In November of 1995, McClellan Integrated Research was finished with the production of the hardware prototype, The project group traveled to the company to lay the tubeset on and make the necessary final adjustments. Changes were made which entailed shortening or lengthening of tubing segments. The changes were necessary because alterations to the hardware prototype were made by McClellan after the mock - up model was manufactured.

Final Tubing Results

Name	Length (in.)	Outer Diameter
A1	5	4.1 mm
A2	5	4.1 mm
A3	9	4.1 mm
A4	3	4.1 mm
A5	1	4.1 mm
A6	15.5	4.1 mm
B6	5.5	4.1 mm
C1	9	4.1 mm
C2	3	4.1 mm
C3	20	4.1 mm
C4	9	4.1 mm
C5	9	4.1 mm
C6	25	4.1 mm
D2	18	4.1 mm
D3	6	4.1 mm
D4	18	4.1 mm
D5	6.5	4.1 mm
D7	17	4.1 mm
D8	6	4.1 mm
D10	6	4.1 mm
E1	9	4.1 mm
B1	5	6.8 mm
B2	4	6.8 mm
B3	11.5	6.8 mm
B4	7	6.8 mm
B5	1	6.8 mm
B7	16	6.8 mm
D1	21	6.8 mm
D9	19	6.8 mm

Once the final design was created, prototype tubesets were sent to the Naval Blood Research Laboratory in Chelsea, MA for testing. Testing with blood verified that the integrity of the tubeset was maintained throughout the washing process. Thus, the integrity testing performed on the tubesets was an adequate

and successful method of testing. Testing performed with blood also proved the baffled process bag worked properly at the modified height. Based on the fact the testing proved the tubeset worked, the final design was achieved and production of the 50 tubesets commenced.

The results of the development of the tubeset lie in the form of tubing and component configurations. These configurations are referred to as subassemblies and are represented in this section as figures. The subassemblies are then integrated and the final prototype is produced.

5.1 Sub-Assembly 1 Figure 5.1: Subassembly 1

5.2 Sub-Assembly 2 Figure 5.2: Subassembly 2

5.3 Sub-Assembly 3 Figure 5.3: Subassembly 3

5.4 Sub-Assembly 4 Figure 5.4: Subassembly 4

5.5 Sub-Assembly 5 Figure 5.5: Subassembly 5

5.6 Sub-Assembly 6 Figure 5.6: Subassembly 6

5.7 Sub-Assembly 1 Figure 5.0: Tubeset Layout

Figure - 5.0
TubeSet Layout

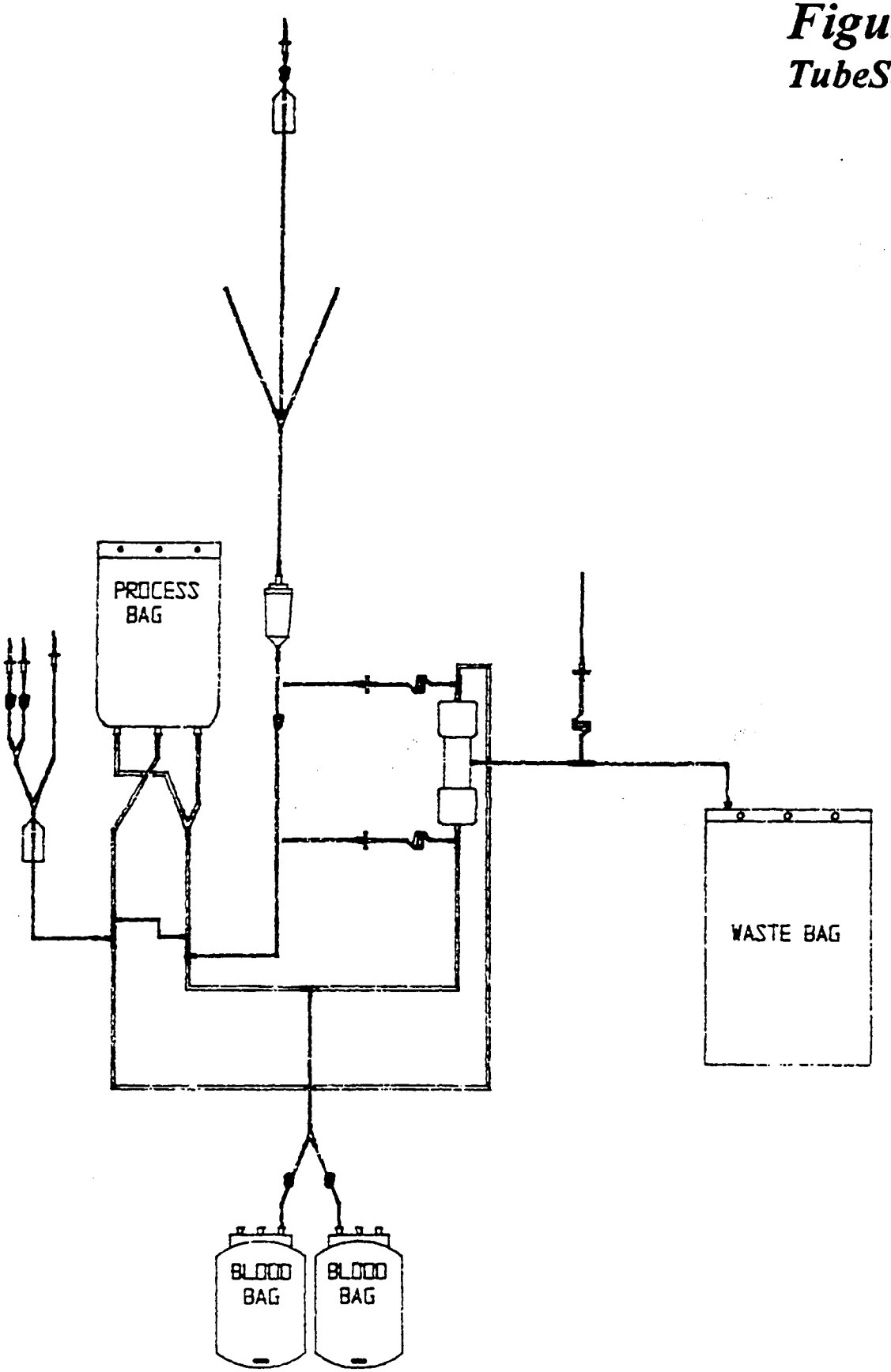


Figure - 5.1
Subassembly1

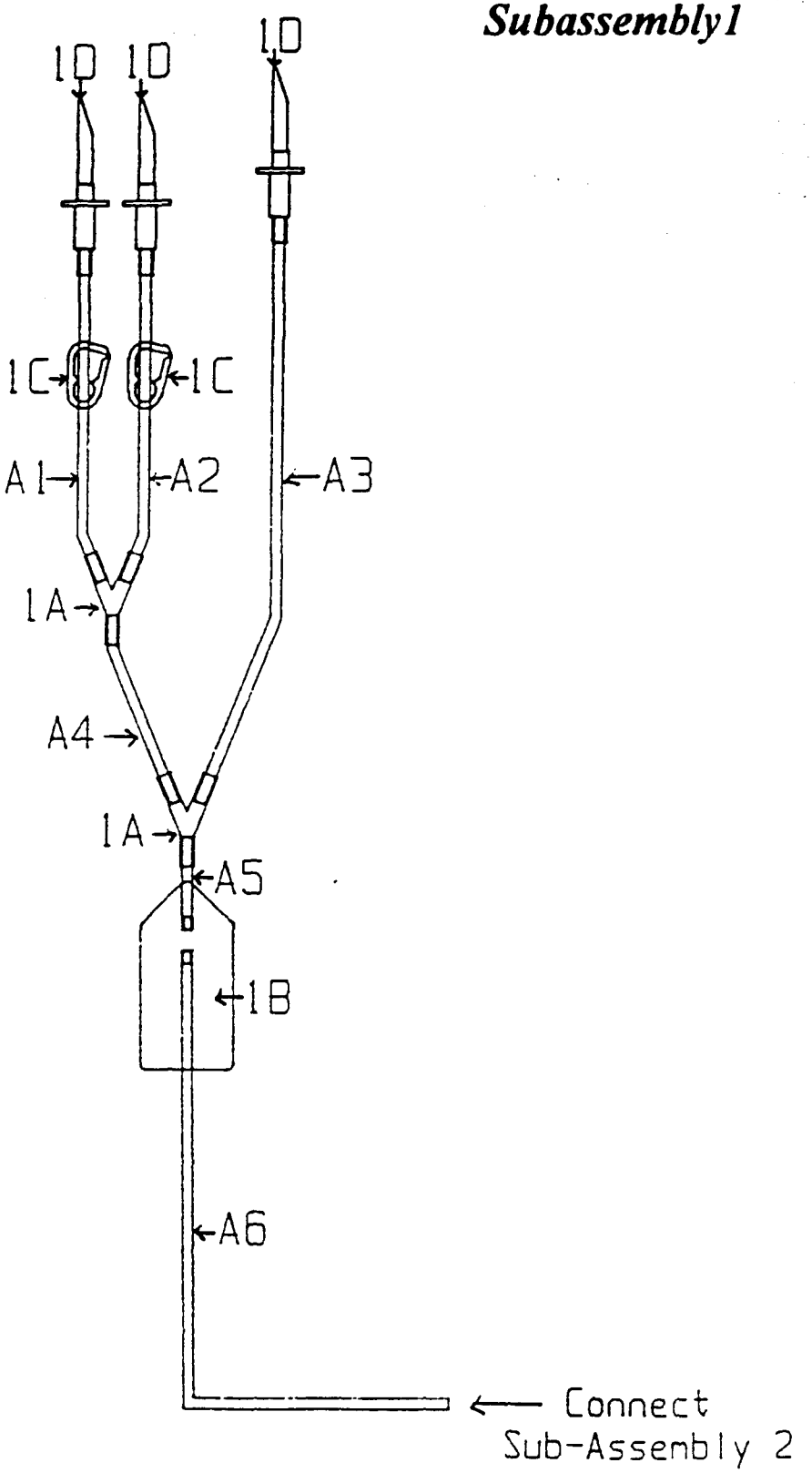
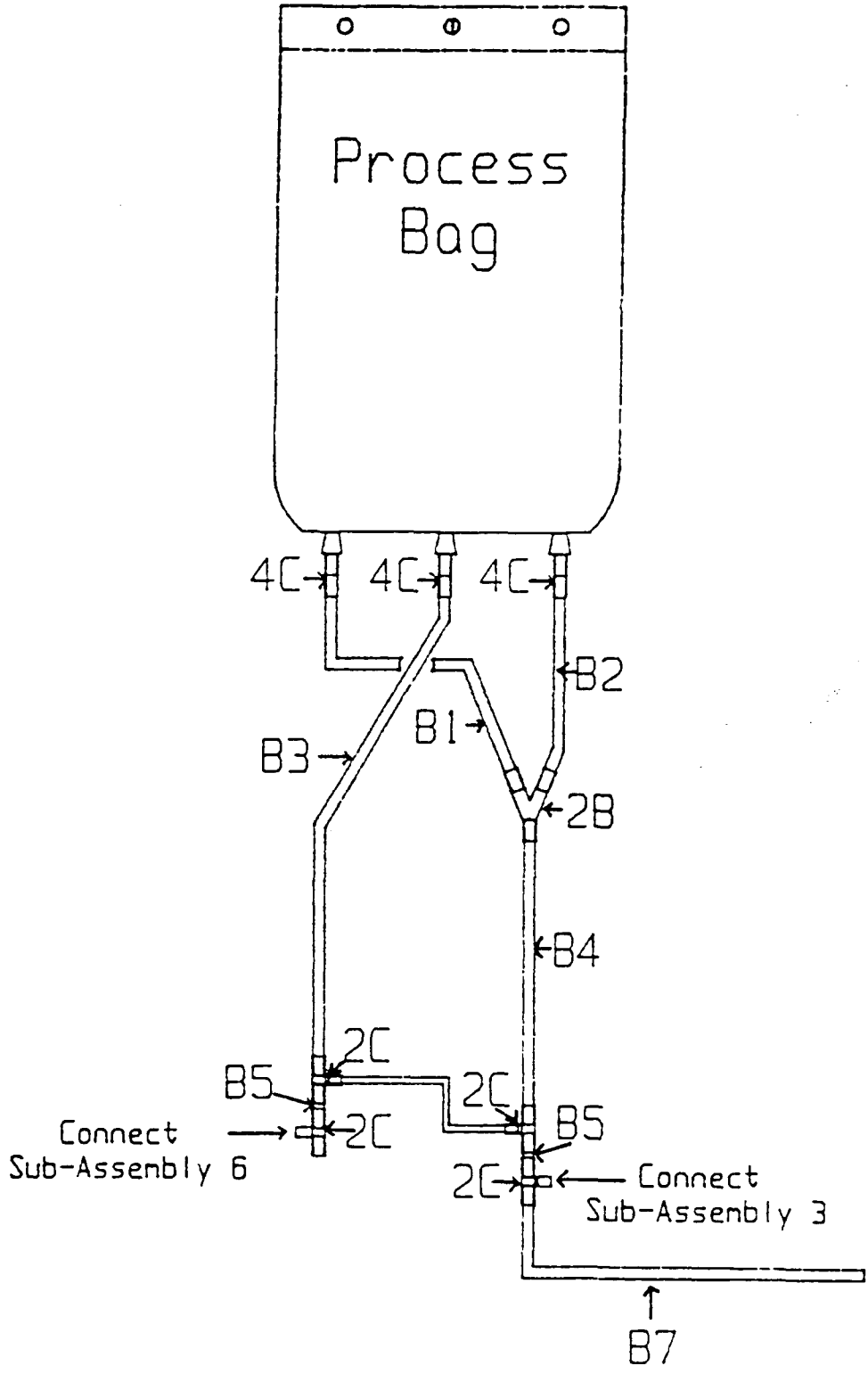


Figure - 5.2
Subassembly2



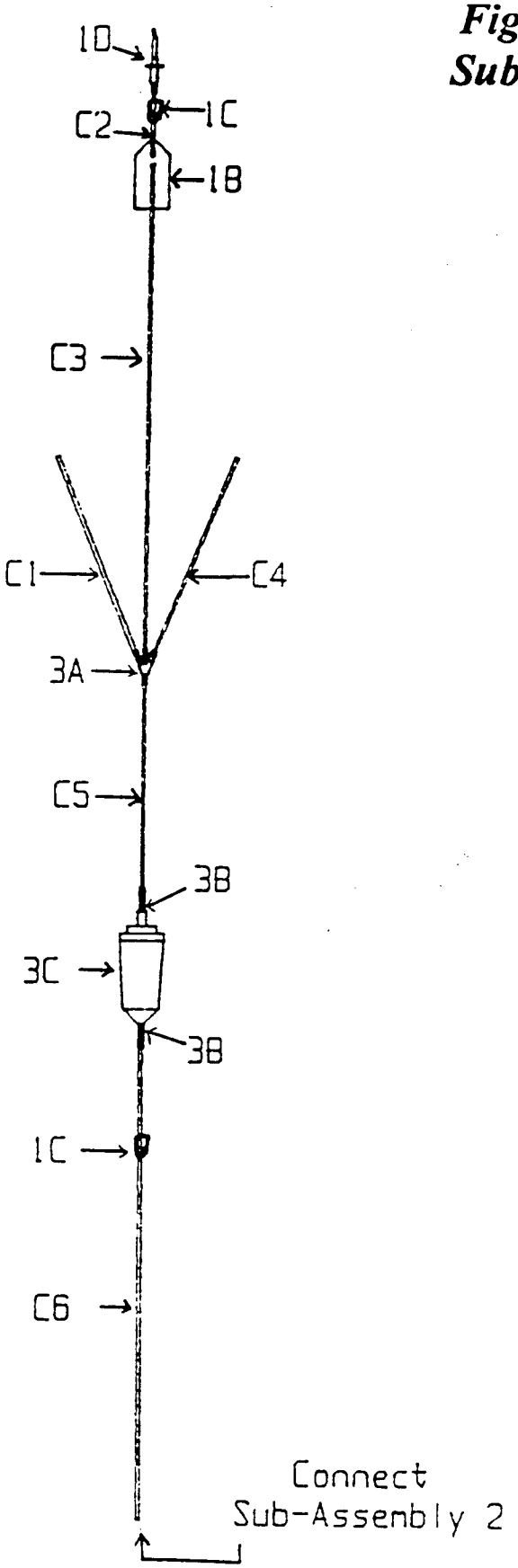


Figure - 5.3
Subassembly 3

Figure - 5.4
Subassembly 4

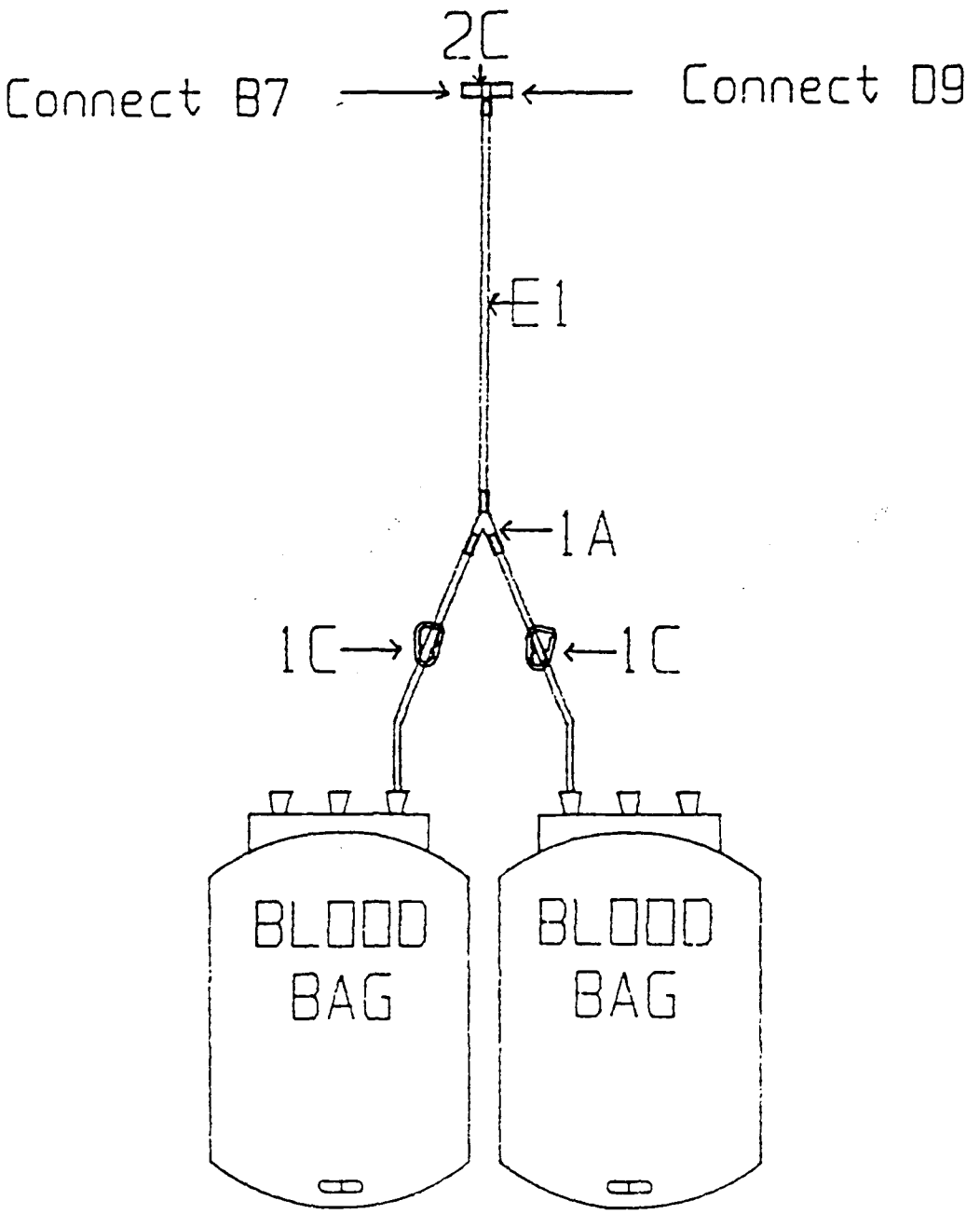


Figure - 5.5
Subassembly 5

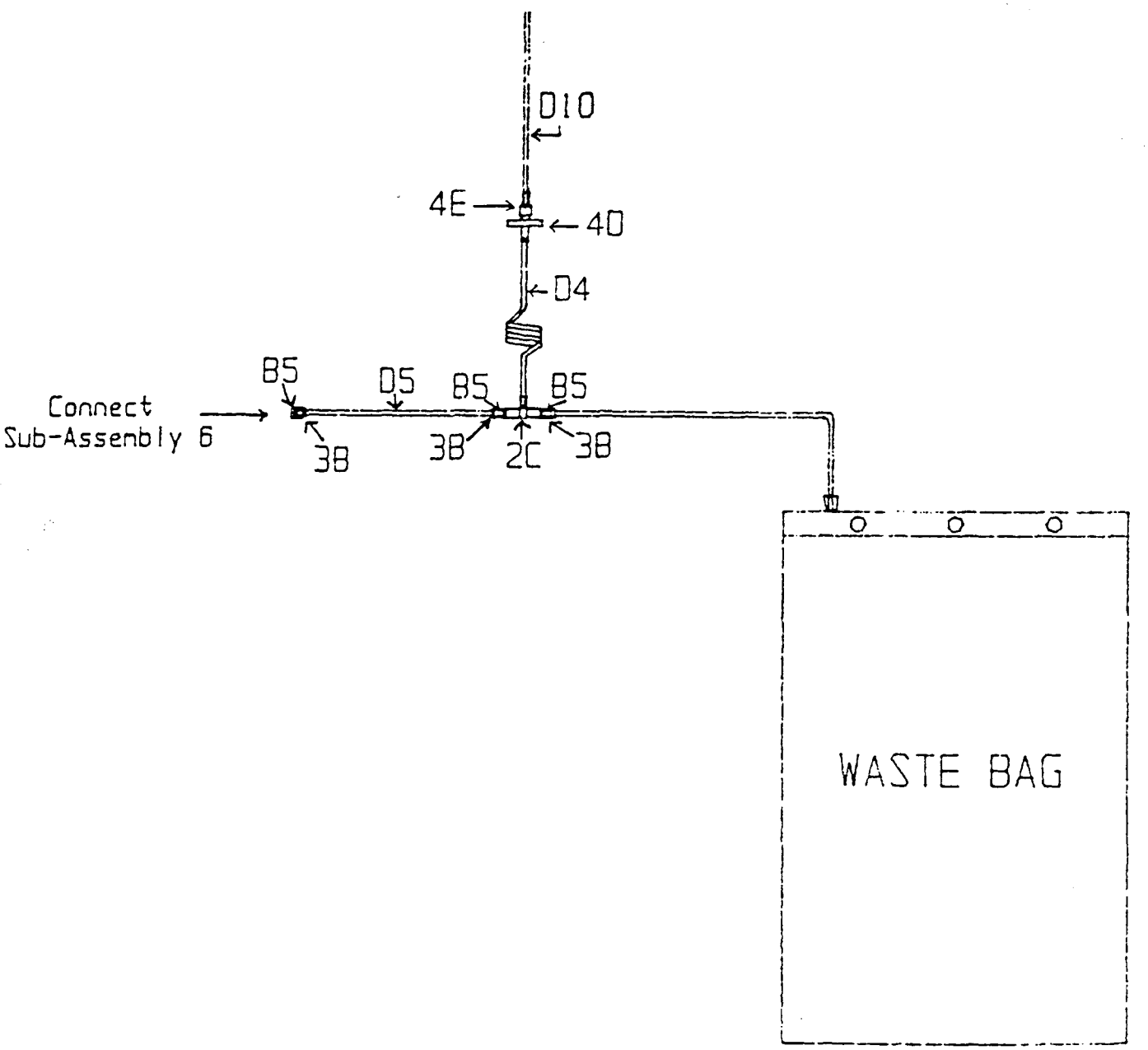
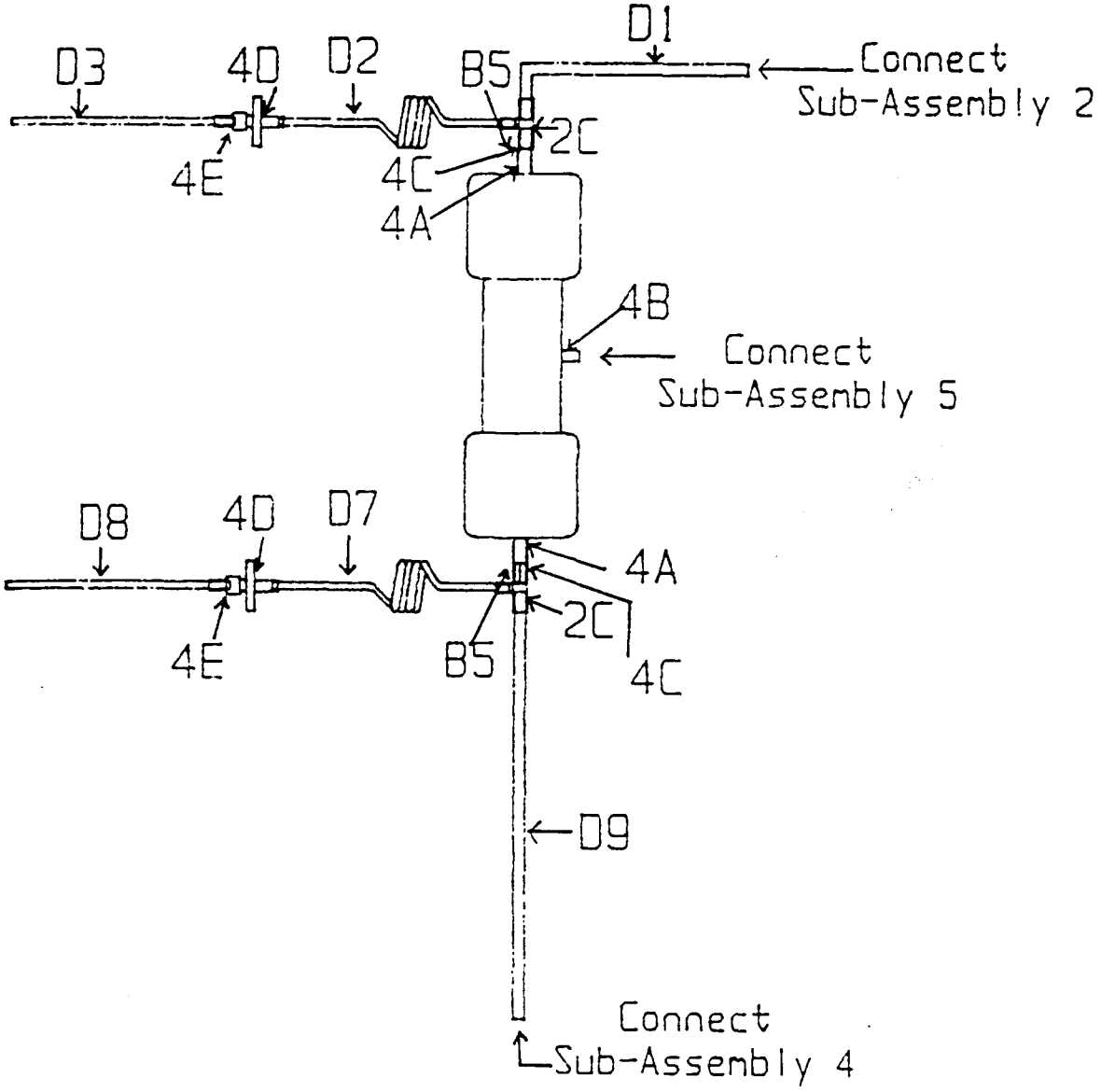


Figure - 5.6
Subassembly 6



DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 19 Apr 00

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCA, 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports. Request the limited distribution statement for Accession Document Numbers ADB210895, ADB223532, ADB209674, ADB231094, and ADB249633, be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Virginia Miller at DSN 343-7327 or by email at Virginia.Miller@det.amedd.army.mil.

FOR THE COMMANDER:

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management